Original Research Article

Antioxidant Potential and Anti-sickling Activity of Different Organs of *Curcuma longa*: Correlation of The Antioxidant Capacity on Anti-sickling Activity

ABSTRACT

Aims: The aim of this research was to evaluate comparatively the anti-sickling activity of aqueous and methanol extracts of different organs of *C. longa,* to determine the polyphenolic compounds and to evaluate the antioxidant potential of different parts of *C. longa*. Also, to establish a possible correlation between the antioxidant potential of the extracts on the anti-sickling activity.
Study design: The study used an experimental design. The antioxidant and anti-sickling activities of the aqueous and methanol extracts of different organs of *C. longa* were assessed along with their correlation.
Place and Duration of Study: The study was carried out at the Laboratory of Natural Products, Faculty of Science and at Centre for the Study of Natural Products from Plant Origin (CESNOV), Faculty of Pharmaceutical Sciences, both at University of Kinshasa in 2018.
Methodology: *C. longa* was planted in an experimental monoculture setting from where were collected different organs of this plant. After processing these parts, they were macerated in methanol and water respectively. After filtration of the macerates, the filtrates were dried in the oven to obtain aqueous and methanol extracts which were used for biological analyses and the determination of polyphenolic compounds. This determination was performed using a spectrophotometer while the biological activities was performed using the ABTS, DPPH and Emmel tests respectively. Data were analyzed using GrapPad Prism 6.0 software for statistical analyzes and the determination of IC_{50}.
Results: The findings showed that the polyphenolic compounds are not distributed in the same way in different organs of *C. longa*. The extracts of different parts of *C. longa* do not all have a great antioxidant potential. However, the rhizome and root extracts showed a better anti-scavenging activity using ABTS method compared to other organs, while rhizome and leaf extracts showed better anti-scavenging activity using the DPPH test. The analyses carried out revealed: a positive correlation between (i) the total polyphenol contents and the anti-sickling activity of the studied extracts (r = 0.08328) and (ii) between the antioxidant activity using ABTS and DPPH tests along with the anti-sickling activity of different extracts (r = 0.1221, 0.4900 and 0.3114 respectively).
Conclusion: The tested extracts displayed good antioxidant and anti-sickling activities. Thus, different organs of *C. longa* are a source of natural antioxidants, which can be used to manage sickle cell disease. The effect of the antioxidant potential of the extracts tested on their anti-sickling activity was demonstrated. Nevertheless, in-depth studies are required in order to confirm this correlation.

Keywords: *Curcuma longa,* Antioxidant activity, Anti-sickling activity, Polyphenol content, Correlation

1. INTRODUCTION

Sickle cell disease is a genetic disease with autosomal recessive inheritance linked to an abnormality of hemoglobin precisely hemoglobin S. At the molecular level, it is a hemoglobinopathy resulting from a point mutation at the sixth codon of the β-globin gene carried by chromosome 11. In fact, it is a substitution of Adenine by Thymine at the 6th codon in the β-globin gene. At the protein level, this mutation leads to the replacement of glutamic acid (polar amino acid) at the 6th position by a valine (apolar amino acid) (β6 Glu → Val), thusthe synthesis of an abnormal hemoglobin, hemoglobin S (Hb S), in English "sickle" [1-4]. Sickle cell disease is responsible for the loss of life worldwide, and the most vulnerable layer is children under 5 years old [5]. From its regions of origin (Central and West Africa), it has moved and continues to move with migratory flows (Mediterranean region, Asia, USA, the Caribbean). However, the African populations are particularly affected. In some West African countries, 30-40% of the population is carrier this gene [3]. Several treatment modalities have been

considered namely the bone marrow transplantation, gene therapy, blood transfusion and the hydroxyl-urea intake in order to relieve patients. The truth is that these treatments are not only ineffective but very costly for poor people in Africa, but can also be the cause of several adverse effects (toxicity, blood incompatibility, graft versus host reaction, HIV/AIDS contamination) [5-7]. Due to all the known side effects, medicinal plants are used in Africa to relieve several ailments In the Democratic Republic of the Congo (DRC), the phytotherapy is currently used as an alternative, which can provide a relief to sickle cell patients. The review of the literature indicated that the screening of several plants have been revealed a potential anti-sickling. Among these plants were also mentioned *Curcuma longa* [2, 5-9, 61-69].

Belonging to Zingiberaceae family, *C. longa* L. is a perennial herbaceous plant measuring up to 1m high. It is mostly cultivated in the tropics but a large part of the production is performed in India and Southeast Asia [9-10]. Several studies reported that the intense cultivation of *C. longa* is due to the use of its rhizomes. For centuries, *C. longa* rhizomes were efficiently used in various sectors including the food, cosmetology, agri-food, pharmaceutical, textile and traditional medicine industries [9-38]. Furthermore, its leaves are used for the extraction of essential oils and the seasoning of dishes or as a colouring agent in the industry and in the traditional kitchens respectively.. In traditional medicine, these leaves are used as tonic, stimulant and blood purifier in the valley districts of Manipur, India [23, 28, 34-36]. Mbadiko *et al.* [9] and other researchers [20, 26, 31, 34, 54-57], reported 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, protects against diabetic retinopathy and numerous other pathologies. However, other studies have shown that the pharmacological actions reported on rhizome extracts of *C. longa* are linked to the presence of polyphenolic compounds, particularly curcuminoids notably curcumin, but also to its essential oils [8-10, 12, 23-24, 39-40, 41-53]. Ajay *et al.* [24] and Camatari *et al.* [17], showed that *C. longa* flowers possess the antioxidant potential. In addition, Lassa [58] reported that *C. longa* rhizomes is used for the treatment of SS anemia in traditional medicine in DRC. This use was scientifically justified by Mbadiko *et al.* [9] whom showed the anti-sickling potential of total methanol extracts from different organs (rhizomes, roots, leaves, sepals and petals) of *C. longa*. Henceforth, the interest to carry out a comparative study on the anti-sickling activity of methanol and aqueous extracts of *C. longa* different organs.

By the way, several reports have showed the involvement of oxidative stress in sickle cell anemia. Hierso [1] reported that sickled red blood cells produce more free radicals than normal red blood cells, this leads to the increase of the production of oxidative biomarkers, which would actively participate in the physiopathology mechanisms of the disease. Then, it plays a role in the hemolysis and the onset of vaso-occlusive crises as well the attacks of various organs. Due to oxidative damage associated with sickle cell disease, the development of therapeutic antioxidant strategies is proven essential and relevant. Henceforth, the aims of this study were to (i) evaluate the antioxidant potential of different parts of *C. longa* and (ii) look for a possible correlation between the content of polyphenolic compounds, the antioxidant potential and the anti-sickling activity of the extracts. Studies raising from this perspective have not yet been reported in the literature.

2. MATERIALS AND METHODS

2.1 Biological material

Different organs of *C. longa* (rhizome, root, leaf, petal and sepal) were used as biological material. The plant was first cultivated as a monoculture in an experimental setting and different parts were harvested. These organs brought to the laboratory where they were washed, dried in the oven (Melag Nur Für Wechselstrom brand) at 30 °C and crushed using a Moulinex electric mill. Different powders obtained were used for the preparation of different extracts that were used for the phytochemical screening and the evaluation of biological activities.

This study was carried out at the laboratory of Natural Products, Department of Chemistry and at the laboratory of Analyses and Research on Food and Nutrition (LARAN), both at Faculty of Science and at Centre for the Study of Natural Products from Plant Origin (CESNOV), Faculty of Pharmaceutical Sciences, both at University of Kinshasa in 2018.

2.2. Determination of Total Phenolic content

The dosage of the polyphenolic compounds was carried out according to the method described by Kapepula *et al.* and Adepado *et al.* [59-60]. In fact, 10 mg/mL of each extract were diluted in methanol 80% in order to obtain a solution of 1mg/mL for each extract. Then, a mixture consisted of 0.5 mL of the extract; 5.0 mL distilled water and 0.5mL of Folin-Ciocalteu reagent was prepared for each extract. After three minutes, 1.0 mL of a saturated solution of Na_2CO_3 20% was added. The mixtures prepared were stirred and incubated at laboratory temperature under shade for one hour. After the incubation, different absorbances were read using a spectrophotometer at 725 nm. Each analysis was performed in triplicate. The quantity of total polyphenols was expressed in mg of gallic acid equivalents (GAE)/g using the following equation from the calibration line:

$$y = 1.7097 \ln(x) + 5.2062 \quad [1]$$

and R^2=0,965, where x is the absorbance and y is the equivalent of gallic acid (mg/g).

2.3. Dosage of total flavonoids

The estimation of the total flavonoid content of our extracts was performed using a spectrophotometer. Aluminium trichloride forms a yellow complex with flavonoids that absorbs at 415 nm [60]. First, a mixed reaction containing 1mL of the methanol solution (80%) of each extract (concentration 10mg/mL) and 1ml of $AlCl_3$ (2%) (dissolved in methanol) was prepared. The mixtures were then stirred and incubated for one hour at the laboratory temperature under shade and the absorbances were read at 415 nm.. For the preparation of the control, the process was the same as described above except that instead of using the extract, 1 mL of methanol was added. Each analysis was performed in triplicate. The flavonoid content of the extracts was expressed in mg quercetin equivalent (QE)/g using the following equation from the calibration line:

$$y = 0.5001 \ln(x) + 3.442 \quad [2]$$

R^2 =0,944, where x is the absorbance and y is the equivalent of quercetin (mg/g).

2.4. Determination of total anthocyanins

The determination of the anthocyanin content of extracts was carried out following the protocol as per Adedapo *et al.* [60]. We took 0.5 mL of the 1mg/mL solution of extract prepared with methanol (80%), which was mixed with 3 mL of vanillin methanol solution (4%) and 1.5 mL hydrochloric acid. The mixture was incubated for one hour, then the absorbance was measured at 500 nm. The same procedure was repeated for the control (D-catechin) in the concentration range (from 4 mg/mL to 0.125 mg/mL) and allowed the calibration line to be established. The anthocyanin content of the extracts was expressed in mg equivalent of D-catechin per g of dry vegetable matter (EC/g) using the following equation from the calibration line:

$$y = 0.0728 x + 0,0171 \quad [3]$$

and R^2= 0.994, where x is the absorbance and y is the equivalent of catechin (mg/g).

2.5. Assessment of the anti-sickling activity

2.5.1. Blood samples

The blood samples used in our experiments were collected at the Centre de Médecine Mixte et d'anémies SS (CMMSS) from the sickle cell patients. Once in the laboratory, they were kept in the refrigerator and the Emmel test was performed 24 hours later to ensure that these samples were actually taken from sickle cell patients.

2.5.2. Preparation of dry methanol extracts of various organs of *C. longa*

Using a precision scale (Kern 440-35N), it was weighed respectively 25g of roots and rhizomes, 20g of leaves, 3.69g of sepals and 5g of petals which were macerated in 100 mL of methanol during 24 hours. The macerates were then filtered using a Whatman paper and dried in the oven at 37 ° C (Melag Nurfur Wechselstrom) for five days.

2.5.3. Preparation of dry aqueous extracts of various organs of *C. longa*

With a precision scale (Kern 440-35N), it was weighed respectively 20g of rhizomes and roots, 15g of leaves and 10g of sepals and petals of *C. longa* powders that were diluted in 150 mL of distilled water. After 24 hours of maceration, the macerates were filtered using a Whatman n°1 filter paper, poured into petri dishes and dried in an oven at 40 ° C for a week. The dry extracts obtained were used for the Emmel's test.

2.5.4. Emmel's test

The principle of this test is the following: Hemoglobin S is less soluble when the oxygen content decreases (hypoxia, pO2<45mmHg). In sickle cell disease, the sickling of erythrocytes is observed due to the intracellular crystallization of hemoglobin S in the form of tactoids. Therefore, unlike the normal red blood cell, which has a biconcave or circular shape, the red blood cells of sickle cell patients have a sickle or banana shape under hypoxic conditions. The effect of crude plant extracts, which prevents cell sickling under hypoxic conditions (created by the addition of sodium metabisulfite 2%, m/v), is used as an indication of the pharmacological activity of these plants.

The anti-sickling activity of the aqueous and methanol extracts of different parts of *C. longa* was evaluated using the Emmel's test, as described previously [8-9, 61-69]. Different concentrations of extracts were prepared notably: 1mg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL and 15.62 µg/mL, using saline solution (NaCl 0.9%, m/v) as a solvent. From this solution, we were able to make a microscopic preparation whereby a drop of SS blood was placed on the slide to which was a drop of $Na_2S_20_5$ 2% in order to create a hypoxic condition. Then, a drop of the extract was added to that preparation, which was mixed thoroughly and carefully covered to avoid the formation of air bubbles. Another microscopic preparation not containing the extracts was made under the same conditions and served as a negative control. The edges of the slide was supercooled with paraffin to reinforce the hypoxic solution. The results was read after 24 hours using a microscope (Bresser) at 40 of magnitude. All the analyses were carried out in triplicate.

2.6. Antioxidant activity

The evaluation of the antioxidant activity was performed using the ABTS and DPPH tests as described by Kapepula *et al.* [59].

2.6.1. Preparation of samples

In fact, 10mg of dry extract from each sample was dissolved in 1mL of methanol for polar extracts and dichloromethane-methanol mixture (1:1) for apolar extracts (solution A: 10 mg/mL) respectively. We then diluted to the following concentration: 8mg/mL, 6 mg/mL, 4mg/mL and 2 mg/mL.

2.6.2. Preparation of ABTS radical

In 500 µL of distilled water was dissolved a quantity of the ABTS reagent corresponding to 20 millimoles: solution A. In another 500µL of distilled water was dissolved a quantity of potassium persulphate ($K_2S_2O_8$) corresponding to 10 millimoles: solution B. Both solutions were mixed at the same volume and the mixture was kept under shade between 12 and 16 hours. This mixture constituted the $ABTS^{\bullet+}$ radical stock solution. Thereafter, the stock solution of the radical was diluted several times with methanol to obtain an analytical solution having an absorbance ranging from 0.800 to 1.000.

2.6.3. ABTS test

When reacted with potassium persulfate ($K_2S_2O_8$), ABTS (2,2'-azino-bis-3ethylBenz-Thiazolin-6-Sulfonic acid) forms the cationic radical $ABTS^{-+}$ from blue to green. The addition of the antioxidant reduces this radical and causes the mixture to discolour. The radical discoloration measured by spectrophotometer at 734 nm is proportional to the antioxidant concentration [59]. For each sample and concentration (8mg/mL, 6 mg/mL, 4mg/mL and 2 mg/mL), 20µL of the solution was collected and 1980µL of the $ABTS^{-+}$ radical analysis solution was added later. The reaction mixtures were incubated for 30 minutes and the solutions were successively read with a spectrophotometer at 734 nm. The same procedure was repeated for the preparation of the control consisting of methanol (20µL) and ABTS (1980µL). The analysis was performed in triplicate.

The percentage of inhibition of the $ABTS^{-+}$ radical by the sample is determined using the following formula:

$$\%inhibition = [1 - (\frac{A_x}{A_c})]x\,100 \quad [4]$$

Where: Ax : the absorbance of the ABTS-+ radical in the presence of the extract
Ac: the absorbance of the ABTS-+ radical (control solution)

2.6.4. Preparation of the DPPH radical

A quantity of 3.2 mg of DPPH reagent was dissolved in 100 mL of methanol 80% and this solution was kept under shade for at least one hour. The absorbance of this solution was adjusted to 0.7 ± 0.05 using methanol 80%.

2.6.5. DPPH test

The DPPH test is based on the degradation of the DPPH- (2,2 DiPhenyl-1- PicrylHydrazyl) radical. The DPPH- radical is a violet coloured radical, the addition of antioxidant reduces this radical and causes the discoloration of the mixture; this discoloration of the radical measured by spectrophotometer at 517 nm is proportional to the concentration of antioxidants added [59]. For each sample and dilution level, 20 µL of the solution was taken and 1980µL of the DPPH- radical analysis solution was added. The reaction mixtures were incubated under shade for 30 minutes and the solutions were read successively with a spectrophotometer at 517 nm. We proceeded in the same way for the preparation of the control, except that instead of the extract, 20 µL of methanol was added. As with the ABTS test, the analysis was performed in triplicate.

The percentage of inhibition of the ABTS-+ radical by the sample is determined using the following formula:

$$\%inhibition = [1 - (\frac{A_x}{A_c})] x\ 100 \qquad [5]$$

Where: Ax : the absorbance of the DPPH- radical in the presence of the extract
Ac: the absorbance of the DPPH- radical (control solution)

2.7. Data analysis

The GrapPad Prism 6.0 software was used for data analysis for statistical analyzes and the determination of IC_{50}. For data presented as mean ± standard deviation, we performed the ANOVA one Way test for comparison of sample averages, followed by Tukey's pairwise multiple comparison test. We considered the threshold of significance at α = 0.05. In addition, Pearson's test was used in order to assess the correlation between some variables.

3. RESULTS AND DISCUSSION

3.1. Total Phenolic content

The content of polyphenolic phytoconstituents of different extracts of C. longa is presented in table 1.

Table 1: Content of polyphenolic phytoconstituents in different organs of *C. longa*

Samples	Tota polyphenol content (mg/EAG) (n=3)	Flavonoid content (mg/EQ) (n=3)	Anthocyanin content (mg/EC) (n=3)
Rhizomes	12.82 ± 0.63[a]	0.020 ± 0.001[a]	0.29 ± 0.05[a]
Roots	5.91 ± 0.87[b]	0.010 ± 0.001[b]	0.17 ± 0.04[a,b]
Leaves	4.31 ± 0.68[c]	0.020 ± 0.002[c]	0.30 ± 0.19[a]
Petals	1.53 ± 0.28[d]	0.007 ± 0.000[d]	0.05 ± 0.01[b,c]
Sepals	0.59 ± 0.30[d]	0.006 ± 0.001[d]	0.01 ± 0.00[b,c]

Mean values followed by the same letters in each column do not differ significantly at p ≤ 0.05 by the Tukey test, n= number of repetition of the experiment.

The ANOVA showed that extracts from different organs of *C. longa* showed very highly significant differences (p value <00001) in total polyphenol content. However, the Tukey pair multiple comparison

test (HSD = 0.4938) does not reveal a significant difference between roots and leaves and between sepals and petals. This indicates that the total polyphenol content is low in the sepals and petals. It has the following descending order: Rhizomes> roots, leaves> petals and sepals. This shows that rhizomes are an important source of total polyphenol in *C. longa*. Maizura *et al*. [14] reported that *C. longa* rhizomes are rich in polyphenolic compounds. Although the rhizome samples used in their study had a high content of total polyphenols (67.9 ± 1.0 mg GAE/100g extracts) compared to those used in this study. This may be explained by the fact that these two samples were not collected in the same geographical environment. Gayatri *et al.* [70], reported that the rhizomes of *C. longa* have a high content of total polyphenols compared to other species of *Curcuma* genus.

However, the floral extracts showed low levels of total polyphenols Yan *et al.* [26], Ajay *et al.* [24] and Camatari *et al.* [17] revealed the presence of large amounts of total polyphenols in floral extracts (2013.09 ± 5.13 mg GAE/100g, 210.45 ± 1.32 mg GAE/100 g and 119 mg ± 2 GAE/g respectively). Chang *et al.* [71] also reported that *C. longa* flowers were rich in polyphenolic compounds (including curcuminoids). In fact, the content of secondary metabolites precisely polyphenolic compounds for the current study may vary with respect to the geographical origin, the period of collection, the physicochemical and biochemical characteristics of the soil, to the method and solvents used for the extraction, to the storage and post-harvest treatment conditions, the genotype of the plant and the part of the plant used [12-13, 56, 72-75].

In this study, the post-harvest treatment of floral pieces may have influenced polyphenolic content. Indeed, while drying floral pieces under shade at the laboratory temperature; these organs tend to soften and lose color; which implies a deterioration of pigmentary principles including polyphenolic compounds. Yan et al. [26] and Chang *et al.* [71] have also reported the degradation of plant polyphenolic compounds under cellular conditions i.e. fresh samples.. Moreover, Yan *et al.* [26], reported that dried and powdered *C. longa* leaves have a high total polyphenol content as fresh samples. The author justified this observation by the fact that the degradation of phytoconstituents in cellular conditions probably due to the activation of hydrolases and/or cellular oxidases. Secondary metabolites are synthesized mainly in response to environmental constraints (biotic or abiotic stresses) and intervene in plant-environment interactions. Some secondary metabolites are considered as messenger molecules and are involved in the initiation of certain biochemical and/or physiological processes of the plant. These processes explain the presence or absence of phytoconstituents during a period of the plant cycle or following the environment conditions [76]. In addition, the concentration of certain polyphenolic compounds, especially curcuminoids in the rhizomes of *C. longa*, becomes important five months after planting and declines between the 9th and 10th month [12]. This author also stated that the primary rhizomes are rich in phenolic compounds as well as secondary rhizomes [12].

However, we observed low levels of flavonoids and total anthocyanins in different extracts studied. The ANOVA also showed a very highly significant difference in the distribution of flavonoids in different parts of *C. longa* (p value <0, 0001). The Pairwise multiple comparison using the Tukey test (HDS = 0.0005357) shows that there is no significant difference in flavonoid levels in petals and sepals. The order of flavonoid concentration in the different parts of *C. longa* is as follows: Leaves> rhizomes> roots> sepals and petals. The flavonoid content of these extracts is expressed in mg equivalent of quercetin per g of dry extracts. Meanwhile, the ANOVA revealed a highly significant difference in anthocyanin levels of different extracts (p value = 0.0073). Although the Tukey multiple comparison test (HSD = 0.07237) revealed a significant difference only between rhizomes and sepals, sepals and leaves as well as petals and leaves. This shows that the petals and sepals are poor in anthocyanins. In addition, it was observed that the polyphenolic phytoconstituents studied are not distributed in the same way in different organs of *C. longa*. It should be noted that studies on the determination of flavonoid and anthocyanin content of different organs of *C. longa* are still poor.

3.2. Anti-sickling activity

The anti-sickling activity of aqueous and methanol extracts of different organs of *C. longa* are evident from the findings of this study (Figure 1). The anthocyanin-rich leaf extracts showed a great anti-sickling activity (15.62 µg/mL). According to Mpiana *et al.* [61-68], Tula [69] and Ngbolua *et al.* [7], the anti-sickling activity of plants is generally related to the presence of anthocyanins and organic acids. In fact, anthocyanins interact with the hemoglobin S and compete with the polymerization reaction by inhibiting the erythrocyte sickling process [2, 69]. This could justify the fact that leaf extracts showed at the same time high anthocyanin content, and a strong anti-sickling activity. However, the aqueous

extracts of rhizomes, leaves and sepals showed a high anti-sickling activity compared to methanol extracts. Mbadiko *et al.* [9] also reported the anti-sickling activity of methanol extracts of different organs of *C. longa*.

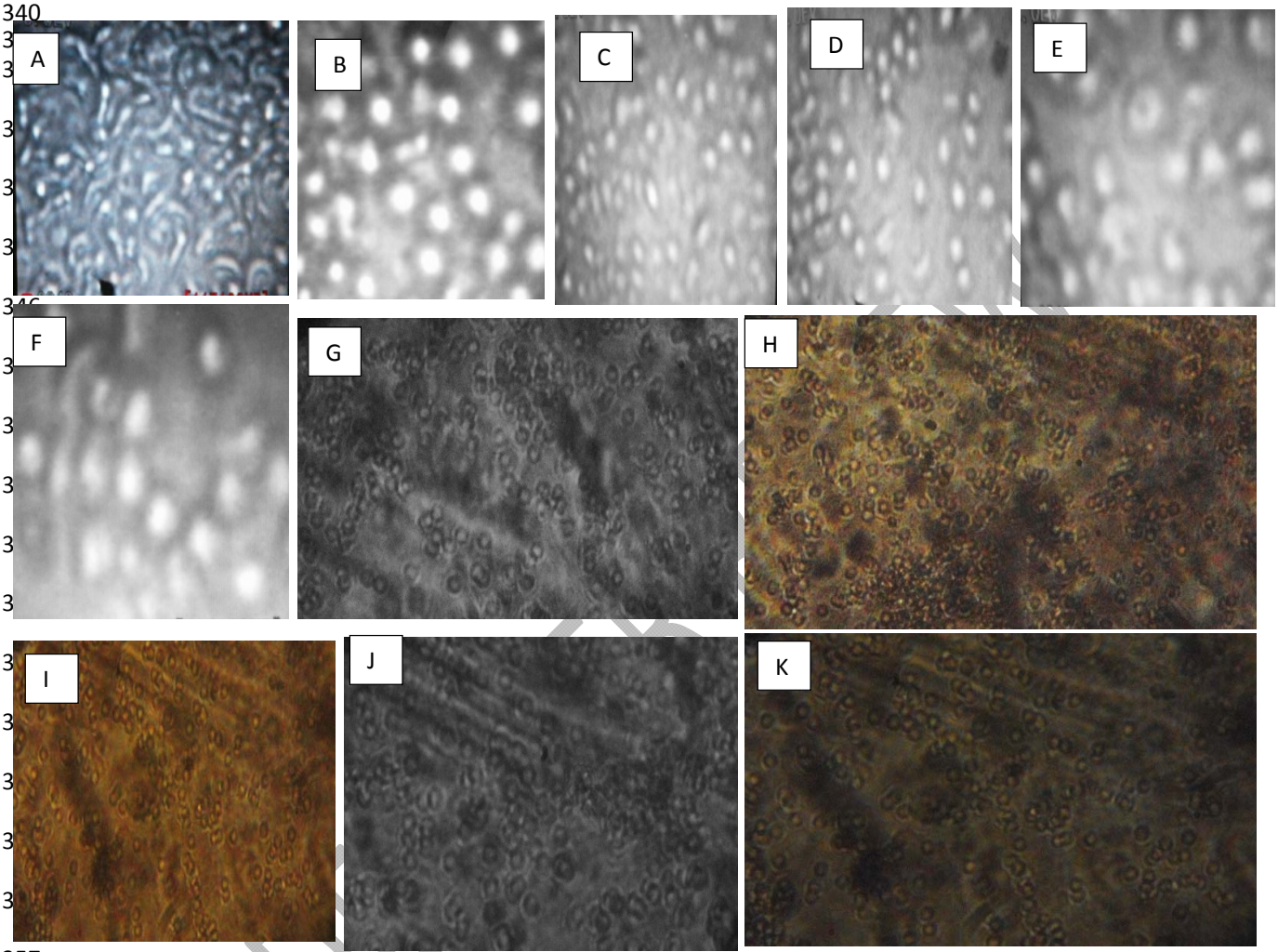

Figure 1. Anti-sickling activity of methanol and aqueous extracts of differents organs of *Curcuma longa* L.

Figure 1 displays the normalization of sickle cells treated with methanol leaf extracts of 31.25 µg/mL (B); rhizomes of 62.50 µg/mL (C), petals of 62.50 µg/mL (D), roots of 62.50 µg/mL (E), sepals of 62.50 µg/mL (F)), and on the other hand, sickle cell treated with aqueous rhizome extracts of 31.25 µg/mL (G), roots of 62.50 µg/mL (H), petals of 62.50 µg/mL (I) , sepals of 31.25 µg / mL (J) and leaves 15.62 µg/mL (K) compared to control, blood SS erythrocytes no treated (A).

The ANOVA showed a very highly significant difference for the anti-sickling activity of methanol extracts of different organs of *C. longa* (p value <0.0001). The Tukey multiple comparison test did not show a significant difference between the activities of rhizome and root, rhizome and sepal as well as rhizome and petal. This reflects the following order in relation to the anti-sickling of extracts from different organs: Leaves > Rhizome, root, petal and sepal. Furthermore, the ANOVA also showed a very highly significant difference for the anti-sickling activity of aqueous extracts of different organs of *C. longa* (p value <0.0001). However, the Tukey Multiple Comparison Test did not reveal a difference between the rhizome and sepal, and then between root and petal. The anti-sickling activity of aqueous extracts follows the following order: Leaves> rhizome, sepal >Petal root.

3.3. Antioxidant activity

The antioxidant activity of different extracts from different organs of *C. longa*, the relationship between the polyphenolic content and the antioxidant activity, and the relationship between total polyphenol content and/or anti-scavenging activity and the anti-sickling activity is presented in the table below.

Table 2: Evaluation of the anti-scavenging activity of methanol extracts from different organs of *C. longa* expressed in IC $_{50}$ by the ABTS and DPPH techniques

Samples	ABTS (µg/mL) (n=3)	DPPH (µg/mL) (n=3)
Roots	4.66 ± 0.34[a]	-
Rhizomes	4.43 ± 0.18[a]	15.63 ± 7.81[a]
Sepals	46.24 ± 2.51[b]	1129.8 ± 559.64[b]
Petals	24.27 ± 1.43[c]	553.35 ± 250.15[c]
Leaves	14.93 ± 4.30[d]	54.83 ± 19.35[d]
Gallic acid (Control)	0.71 ± 0.08	1.07 ± 0.10

Mean values followed by the same letters in each column do not differ significantly at p ≤ 0.05 by the Tukey test, n= number of repetition of the experiment.

The ANOVA revealed a very highly significant difference regarding the antioxidant activity (ABTS test) of *C. longa* extracts (p value = 0.0001). The Tukey pair multiple comparison test (HSD = 0.5365) does not reveal a significant difference between rhizome and root extracts. The intensity of anti-scavenging activity using the ABTS test and follows the following descending order: Rhizomes, roots > leaves > petals > sepals. However, the ABTS method helps to evaluate the antioxidant activity of the substance mixtures but also to distinguish their additive and synergistic effects. Because the radical ABTS reacts at the same time with the lipophilic and hydrophilic compounds (in particular the polyphenols) while the DPPH reacts only with the polar or hydrophilic compounds [59, 77]. Thus, the fact that the rhizomes and roots of *C. longa* showed better activity with the ABTS test proves that these organs have a high content of lipophilic (non-polyphenolic compounds) and hydrophilic (polyphenolic compounds) compounds responsible for the antioxidant activity. It could also demonstrate that the anti-scavenging activity of rhizome and root extracts is related to the additive or synergistic effect of polyphenolic and non-polyphenolic compounds. The ANOVA on the antioxidant activity of extracts of different organs of *C. longa* using DPPH test showed a very highly significant difference in the antioxidant activity of these organs (p value= 0, 0001). However, the Tukey multiple-pair comparison test indicated that there is no significant difference between rhizome and leaf extracts. The anti-scavenging activity of extracts from different parts of *C. longa* follows the following descending order: Rhizomes> leaves > petals > sepals. It seems that the antioxidant activity of *C. longa* leaves is largely related to polyphenolic compounds because the DPPH radical reacts particularly with hydrophilic compounds i.e. polyphenols [59, 77].

In addition, for the same sample, the analysis of the antioxidant activity using the ABTS test showed in most cases lower IC_{50} values than those of the DPPH test. This asserts the hypothesis on the additive or synergistic effect of polyphenolic and non-polyphenolic compounds in the antioxidant activity of extracts studied and revealed that non-polyphenolic compounds contribute significantly to their antioxidant property. Maizura *et al.* [14] also reported the synergistic potential of the polyphenolic and non-polyphenolic fraction on the antioxidant potential of plant extracts. Several studies have shown that rhizome extracts of *C. longa* exhibited an antioxidant activity; and this antioxidant potential is mainly due to the presence of polyphenolic compounds, like curcuminoids. [12-13, 15-16, 18, 27, 32-33, 37-39, 49, 51, 73, 78-86]. According to these authors, curcuminoids notably Curcumin is ten times more antioxidant than Vitamin E.

By the way, Jelena *et al.* [28], reported that the essential oils extracted from *C. longa* rhizomes also have a very strong antioxidant activity. There is little information in the literature on the antioxidant

activity of root, leaves and floral extracts. The antioxidant activity of *C. longa* leaf, roots and flower extracts was reported as well by Ajay *et al.* [24], Camatari *et al.* [17] and Ritwiz *et al*. [54].

The comparison of total polyphenol content and the antioxidant activity is presented in table 3 below.

Table 3: Comparison of total polyphenol content and antioxidant activity of different organs of *C. longa.*

Plant Extracts	Total Polyphenol Content (mg/EAG) (n=3)	Antioxidant activity	
		ABTS (µg/mL)	DPPH (µg/mL)
Rhizomes	12.82 ± 0.63	4.43 ± 0.18	15.63 ± 7.81
Roots	5.91 ± 0.87	4.66 ± 0.34	-
Leaves	4.31 ± 0.68	14.93 ± 4.30	54.83 ± 19.35
Petals	1.53 ± 0.28	24.27 ± 1.43	553.35 ± 250.15
Sepals	0.59 ± 0.30	46.24 ± 2.51	1129.80 ± 559.64

The correlation between the total Polyphenol content and the antioxidant activity of ABTS and DPPH test of different extracts was evaluated using the Pearson test. The results obtained highlighted the existence of a negative correlation between the concentration of total polyphenols and the antioxidant activity of different extracts using the ABTS test (r = -0.7807) but yet this correlation is not significant. The analyses also showed a negative correlation between the concentration of total polyphenols content and the antioxidant activity using the DPPH test (r = -7445). The non-significant negative correlation between total polyphenol content and the *in vitro* anti-scavenging activity of the ABTS and DPPH test indicated that the antiradical activity of the studied extracts was not related solely to the presence of the polyphenol compounds. Nevertheless, many studies have reported a positive correlation between the total polyphenol concentration of plant extracts and their antioxidant potential [13-14 17, 24, 26, 70, 75]. Shan *et al.* [88] and Wu *et al.* [89], have also reported that phenolic compounds contribute significantly to the antioxidant activity of plant extracts. Velioglu *et al*. [90] and Kaur *et al.* [91], have also shown that there is a linear correlation between the total polyphenol content of plants and their antioxidant potential.

The relationship existing between the polyphenol content, antioxidant and anti-sickling activities ispresented in the following table.

Table 4: Relationship between polyphenol contents, antioxidant and anti-sickling activities

Extracts from plants	Total Polyphenol Content (mg/EAG) (n=3)	Antioxidant activity		Anti-sickling activity /M.E (MNC en µg/mL)	Anti-sickling activity /A.E (MNC en µg/mL)
		ABTS (µg/mL)	DPPH (µg/mL)		
Rhizomes	12.82 ± 0.63	4.43 ± 0.18	15.63 ± 7.81	62.50	31.25
Roots	5.91 ± 0.87	4.66 ± 0.34	-	62.50	62.50
Leaves	4.31 ± 0.68	14.93 ± 4.30	54.83 ± 19.35	31.25	15.62
Petals	1.53 ± 0.28	24.27 ± 1.43	553.35 ± 250.15	62.50	62.50
Sepals	0.59 ± 0.30	46.24 ± 2.51	1129.80 ± 559.64	62.50	31.25

Legend : MNC: Minimum Normalization Concentration, M.E.: Methanol extracts, E.A.: Aqueous Extract, n= number of repetition of the experiment.

Correlations between the total polyphenol content, antioxidant activity and the anti-sickling activity of the extracts were demonstrated using the Pearson test. The analyses carried out revealed: a positive correlation between (i) the total polyphenol contents and the anti-sickling activity of the studied extracts, in particular methanol extracts (r = 0.08328) and (ii) between the antioxidant activity using ABTS and DPPH tests along with the anti-sickling activity of different extracts, in particular methanol extracts (r = 0.1281 and 0.4900 respectively). However, these correlations were not significant. The positive correlation between total polyphenol contents and anti-sickling activity demonstrated that the polyphenolic compounds would contribute effectively at the same time to the inhibition of hemoglobin S polymerization and to erythrocyte sickling. However, the fact that this correlation (total polyphenol content and antioxidant activity of methanol extracts) was not significant suggests that polyphenolic compounds act in synergy with other active ingredients. Tshilanda [2] and Ngbolua *et al.* [7], reported that anthocyanins have the ability to inhibit the polymerization of hemoglobin S by engaging in a reaction with this protein.

In addition, the analysis of the correlation using Pearson test did not show a correlation between the total polyphenol content or antioxidant activity using ABTS test and the antioxidant activity of aqueous extracts (r= -0.1740, r= -0.1481 respectively). Although the correlation between the antioxidant activity of the extracts using DPPH test and the antioxidant activity of aqueous extracts was positive (r=0.3114). This would predict the involvement of the polyphenolic compounds of *C. longa* and their antioxidant potential on their anti-sickling potential; because the DPPH radical reacts exclusively with the polyphenolic compounds. However, in-depth and especially *in vivo* investigations are necessary to elucidate the implications of plant antioxidants on their anti-sickling potential.

Furthermore, the fact that the results of the present study showed a positive correlation between the antioxidant power of the extracts and their anti-sickling potential would reveal the involvement of the extract antioxidants in the normalization of erythrocytes *in vitro*. Considering these findings, Curcuma is a good candidate for the management of sickle cell disease because it has both anti-sickling and antioxidant activities and because of the involvement of oxidative stress in the pathophysiology of sickle cell disease. According to Ngbolua *et al.* [7], anthocyanins are effective scavengers of free radicals and therefore potent inhibitors of lipid peroxidation and can prevent the lysis of erythrocytes through their antioxidant properties. A phytomedicine based on these compounds could ensure the protection of the membrane in sickle cell patients by supplementing the enzymatic defense systems of the erythrocyte, which are defective in sickle cell patients [1].

Several studies reported that some polyphenolic compounds, notably curcumin extracted from *C. longa* rhizomes have the ability to trap free radicals (superoxide anions, hydroxyl radicals, H_2O_2, nitrite radical, etc.), inhibit or regulate the inducible enzyme for the synthesis of nitrite oxide (NO), iNO-Synthetase (iNOS), prevent lipid peroxidation, allow the chelation of metals, maintain the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase [11,18, 23, 84, 87, 92-94]. Hierso [1] reported that administration of N-acetylcysteine, a non-essential amino acid stimulating glutathione production, would significantly decrease the formation of sickle red blood cells and return them to their biconcave form.

Conclusion and perspectives

In the current study was highlighted the distribution of polyphenolic compounds in different organs (rhizome, root, leaf, petal and sepal) of *C. longa*, evaluated the anti-sickling activity of aqueous and methanol extracts of *C. longa* and their antioxidant potential. The findings showed that the polyphenolic compounds are not evenly distributed in different organs (Rhizome, roots, leaves, floral parts) of *C. longa*. Total polyphenol content was higher in the rhizomes followed by roots, leaves, petals and sepals. The flavonoid content is higher in the leaves followed by rhizomes, roots, sepals and petals, while the anthocyanin content is highly in the leaves then rhizomes and other parts are poor in anthocyanins. Aqueous extracts of rhizomes, leaves and sepals were more active than methanol extracts of the same organs. Moreover, it was also found that different organs of this plant do not all have a good antioxidant potential, rhizome and root extracts showed better antiradical activity using ABTS method compared to other organs, while rhizome and leaf extracts showed better antiradical activity using the DPPH test.

A positive correlation between the total polyphenol content and the *in vitro* anti-sickling activity of the methanol extracts, and on the other hand between the *in vitro* antioxidant activity and anti-sickling activity was observed. In view of the involvement of oxidative stress in the pathophysiology of sickle cell disease, it is necessary to carry out *in vivo* studies not only to prove the anti-sickling activity of the polyphenolic compounds of *C. longa* but also to highlight the involvement of their antioxidant potential in the pathophysiology of this disease.

Studies on the anti-sickling activity of aqueous extracts from different parts of *C. longa* or those analyzing the correlation between total polyphenolic content and/or the correlation between the antioxidant and anti-sickling activities have not been reported yet in the literature. Further studies are required in order to (i) better understand the roles of plant antioxidants (especially polyphenols) in improving the clinical signs of sickle cell disease and their *in vivo* mechanisms of action and (ii) identify the active ingredients responsible for the anti-sickling activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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