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Assessment of the antisickling activity of total methanolic extracts from the rhizomes and roots of *C. longa* and the effect of photodegradation on the antisickling activity

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ABSTRACT:

For centuries, extracts from the rhizomes of *C. longa* L. were used in Asian and African traditional medicine for the treatment of many diseases. Several studies reported that the extracts of the rhizomes of this plant possess a broad pharmacological potential, nevertheless the studies on the antisickling activity have not been carried out yet and biological studies on other organs of the plant, namely roots remain poor or not yet performed. Using Emmel test, the antisickling activity of total methanolic extracts of rhizomes and roots of this plant was performed. In addition, we assessed the impact of photodegradation on the antisickling action of total methanolic extracts from *C. longa* rhizomes and roots. To achieve this goal, some of our samples (rhizomes and roots) were dried in the open (i.e. exposed to the sun) and the other part was dried the laboratory temperature under shade. It is known that curcumin, which is the main active ingredient of turmeric longa, degrades in the presence of the UV rays; which justifies the the hypothesis of this work. This study allowed us to conclude that photodegradation has no impact on the antisickling activity of the extracts and all the extracts (total methanolic extracts from rhizomes and roots) showed a good antisickling activity. We suggest further studies that can identify the active compounds responsible of the antisickling activity of *C. longa* rhizomes and roots as well as to demonstrate their mechanism of action.

Keyword: *Curcuma longa*, Antisickling activity, Emmel test, Photodegradation.

INTRODUCTION

Curcuma domestica Val or *Curcuma longa* L. is a plant that belongs to the same family as ginger, Zingiberaceae. It is distributed in the tropical and subtropical regions of the world and is widely grown in Asian countries, namely : Malaysia, Indonesia, India, and Taiwan. The plant is also cultivated in some African countries, precisely in the Democratic Republic of the Congo [1-5] and it is cultivated more for its rhizomes. Once the rhizomes are dried and reduced to a powder, it can be used as a food spice in order to enhance the flavor of foods and preserve them, but also as a coloring agent for food and textiles. They have been used for at least 4000 years in the traditional Ayurvedic popular medical system and is considered as a symbol of prosperity and good health. It also has a long tradition in Chinese medicine [1-5]

C. longa rhizomes are used in the treatment of respiratory diseases (asthma, allergy, bronchial hyperactivity, lung problems), liver and gall-bladder disorders (jaundice), for its carminative properties and for its anti-inflammatory properties. In Chinese traditional medicine, they are used to treat abdominal pain and anorexia [1-5]. In recent years with the rise of chronic inflammatory diseases, cancers, Alzheimer's disease, the western world has become more and more interested in this spice. In fact, it was found that colon cancer is less common in countries where *C. longa* rhizome *C. longa* is consumed daily [3].

Several studies justified and/or demonstrated the *in vitro* and *in vivo* activity using animal models and humans that *C. longa* rhizomes extracts possess a broad pharmacological potential: anti-cancer, anti-inflammatory, cicatrizing, hypocholesterolemic, hypoglycemic, anti-inflammatory, antioxidant, antibacterial, anti-fungal, antivenom, antipyretic, analgesic, inhibits the action of HIV-1 integrase, and replication of HIV-1 integrase protein, protects against Diabetic retinopathy and numerous other pathologies [6-10]. Nevertheless, up to our knowledge no study reported yet on the antisickling activity of *C. longa* rhizomes. This motivated us to assess the antisickling activity of *C. longa* rhizome and root extracts.

Moreover, numerous data from the literature report that the pharmacological properties of *C. longa* are related to the presence of Curcuminoids, in particular curcumin. These compounds are responsible for the yellow color characteristic of the rhizome powder of *C. longa* [3]. Curcuminoids, like certain phytoconstituents degrade when exposed in the presence of UV-visible light [5]. Henceforth, in the present study, we also assessed the effect of photodegradation on the antisickling activity of the extracts of rhizomes and roots of *C. longa*.

2. MATERIALS AND METHODS

2.1 Plant material

We used as plant material: the rhizomes and roots of *C. longa*. The plant was identified at the Hebarium of the National Institute of Agricultural Studies and Research (INERA) at the Faculty of Sciences of the University of Kinshasa.

2.2. Sample collection and packaging

First, the plant was cultivated as a monoculture in an experimental garden and the various organs were then collected. Usually, rhizomes and roots are collected at the end of the plant development cycle i.e. after drying of the plant aerial parts (leaves). We proceeded in different ways to dry our samples. A part of the sample (rhizomes and roots) was dried at the laboratory temperature (under shade), while the other part was dried in the open air (i.e. exposed to the sun) in order to assess the effect of the photodegradation on the antisickling activity.

The fresh sample for each part was weighed (400 g) (roots and rhizomes) using a calibrated balance (Kern 440-35N) and then were arranged to get dried. In order to increase the surface area of contact with heat and reduce the drying time, the fresh samples were previously cut into small pieces. Drying rhizomes at the laboratory temperature can go up to a week (i.e. 7 days) while the drying time of rhizomes and roots dried under the sun was of 4 days.. After drying, the various organs were ground with an electric grinder.

2.3 Obtaining total methanolic extracts

2.3.1 Extraction of active principles

We have weighed 25g of roots and rhizomes using a calibrated balance (KERN 440-35N), which was macerated in 100 ml of methanol for 24 hours. The macerates were filtered with a filter paper (Whatmann n°1) and dried in an oven (MELAG NURFUR WECHSELSTROM) for 5 days.

2.3.2 Yield of the crude extract

The yield of the crude extract is defined as the ratio between the mass of the dry extract obtained and the mass of the treated plant material. This yield is calculated using the equation:

$$\%R = \frac{Me}{Mv} x100$$

% R : Yield in percentage.
Me : Mass of the extract after evaporation of the solvent.
M : Mass of plant material used for extraction [23]

Figure 1. Photos of *C. longa* rhizomes and roots

2.4 Assessment of antisickling activity

2.4.1 Biological material

The blood samples used in our experiment were taken from Centre de Medecine Mixte et d'Anémie SS (CMMSS) in sickle cell patients. Once in the laboratory, the blood samples obtained were kept in the refrigerator. After 24 h, Emmel test was performed in order to be sure that these samples were really taken from sickle cell patients.

2.4.2 Emmel Test

The antisickling activity of total methanolic extracts of different parts of *C. longa* was evaluated by Emmel test, according to the method described by different authors previously [12, 14-20, 22]. In order to evaluate the antisickling activity, different concentrations of total methanol extracts were prepared namely 1 mg/ml; 500 µg/ml, 250 µg/ml, 125 µg/ml and 62.5 µg/ml. Five mg of each sample was diluted in 5 ml of the physiological solution (i.e. a final concentration of 1 mg/ml). Then, from this stock solution, successive dilutions were performed in order to obtain the different concentrations of the aforementioned methanolic extracts. Afterwards, a drop of SS blood was taken and placed in a petri dish to which was added a drop of saline solution and all was homogenized. This process makes the blood less concentrated so that the sickle cells might be well observed under the microscope. Therewith, a drop of sickle blood is placed in the middle of the slide and a drop of the solution to be tested was added, then a thoroughly mixture was performed. After mixing, the slide was covered with coverglass carefully in order to avoid air bubbles and the edges of the coverglass were fixed using a supercooled to create a condition of hypoxia. The result reading was done 24h later using a specialized microscope (BRESSER) at 40x of magnification.

Another microscopic preparation containing no extract was carried out under the same conditions and served as a negative control. It should also be noted that these manipulations were carried out twice for the confirmation of the results.

3. RESULTS AND DISCUSSIONS

3.1 Drying yield

C. longa sample drying (rhizomes and roots) at the laboratory temperature or in the incubator (Melag Nurfur Wechselstrom) lasted one week (7 days). For 400 g of oven-dried rhizomes (Melag Nurfur Wechselstrom) at 37 ° C. or at the laboratory temperature, 69.80 g (17.45% of yield) and 77.44 g (19.36 % of yield). On the contrary, for 400 g of the roots dried in an oven or at the laboratory temperature, we obtained 80.92 g (i.e. 20.23% of yield) and 70.12 g (17.53% of yield) respectively (figure 2).

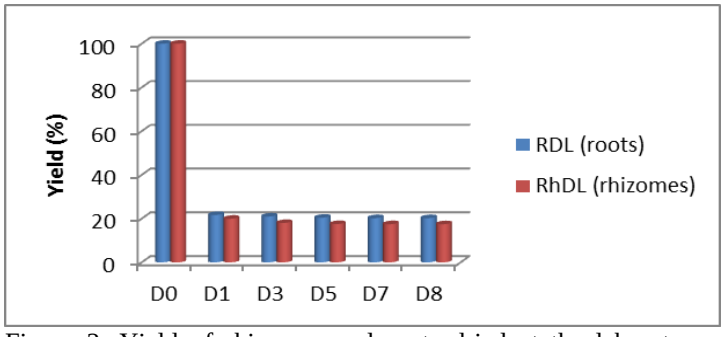

Figure 2. Yield of rhizomes and roots dried at the laboratory temperature

It is shown from figure 3 that that during the drying of *C. longa* root samples, 78.3% of the sample weight was lost after 24h (D1 and it stabilized on D7 and D8).

The drying of rhizomes and roots exposed to the sun varies according to the organs. The duration for rhizomes was of 4 days and of 5 days for the roots. In addition, the yield after drying was 28, 13% for the rhizomes and of 15.9% for the roots respectively (Figure 3).

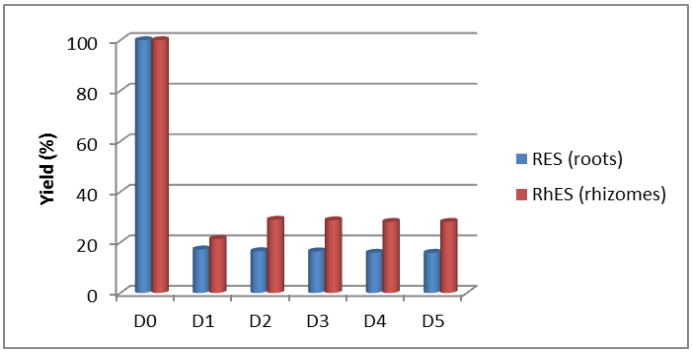

Figure 3. Yield of rhizomes and roots dried under the sun

While drying the rhizomes in the incubator (37 °C), the sample lost 80.03% of its weight at day one, the weight decreased at day two (1.92% of weight lost) and it stabilized at day five.

The study of the daily weight loss allowed us not only to know the yield after drying different samples but also to know the real duration of drying.

3.2 Extraction yield

The total methanolic extracts were obtained by maceration in methanol; the extraction yield was calculated according to the formula given above.

After extraction, for 25 g of rhizome and root powder, we obtained: 0.7 g (i.e. 2.8%); 0.4 g (1.6%); 1.11 g (4.44%) and 0.71 g (2.84%) of the methanolic extracts from the dried roots exposed to the sun, dried roots at laboratory temperature, dried rhizomes exposed to the sun, and rhizomes dried at the laboratory temperature (figure 4). The yield is expressed in percentage.

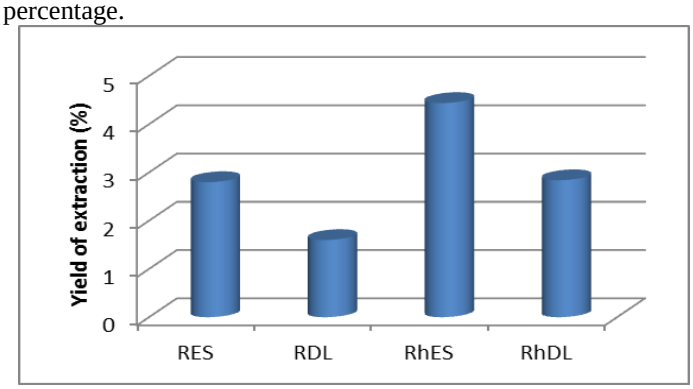

Figure 4: Yield of extraction of rhizomes and roots of *C. longa*

Legend:

- RES: roots of C. longa exposed to sun
- RDL: roots of C. longa dried in the laboratory
- RhES: rhizomes exposed to sun
- RhDL: rhizomes dried in the laboratory

3.3 Test of Emmel

Figures below illustrate the morphology of SS blood erythrocytes and their structure in the presence of the various total methanolic extracts from the rhizomes and roots of *C. longa*.

Figure 4 illustrates that all the cells have a sickle form under conditions of hypoxia, and this confirms that the blood used for analysis is really taken from a sickler patient.

Figure 5. Photo of SS blood erythrocytes no treated

On the other hand, the other figures which illustrate the microscopic preparations containing the extracts of the plant show, under the same conditions, a different morphology of the sickle cell. The cells take back their normal or biconcave form. We believe that these changes result from the presence of total methanolic extracts from *C. longa* rhizomes and roots added to the blood sample during microscopic preparation.

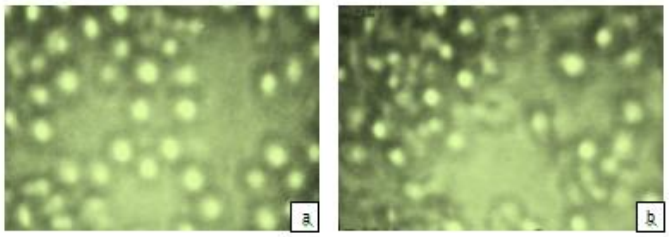

Figure 6: Total methanolic extracts of *C. longa* rhizomes dried under the sun : (a) 1mg/ml and (b) 125µg/ml

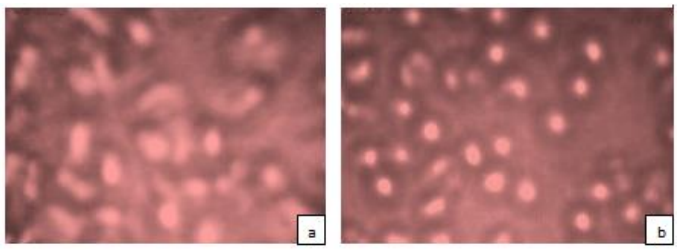

Figure 7: Photos of treated SS blood erythrocytes with total methanolic extract of dried roots exposed to the sun : (a) 125 µg/ml and (b) 1mg/ml

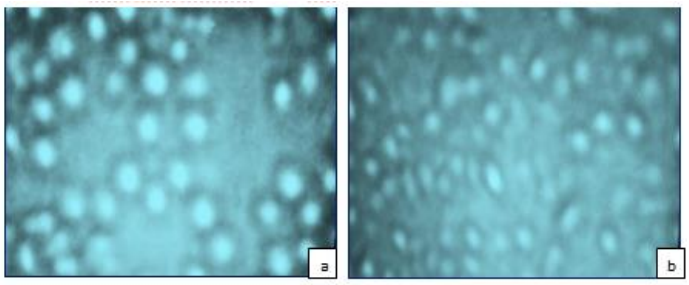

Figure 8: Photos of treated SS blood erythrocytes with total methanolic extracts of rhizomes dried at the laboratory temperature (a) 1mg/ml and (b) 500µg/ml

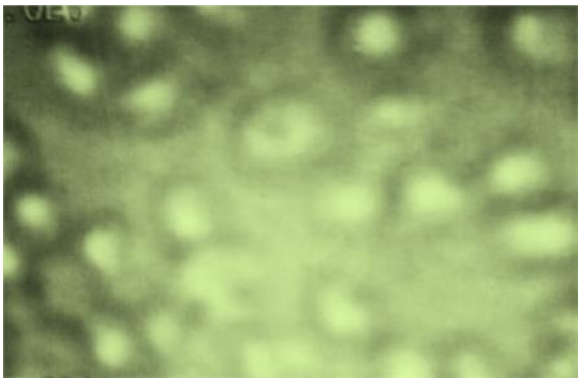

Figure 9: Photos of treated SS blood erythrocytes with total methanolic extracts of roots dried at the laboratory temperature : 500µg/ml

It appears that photodegradation has no real impact on the extracts tested. In fact, rhizomes and roots extracts dried at the ambient temperature (i.e. exposed to the sun) and those dried at the laboratory temperature all showed an antisickling activity. The minimum concentration of normalization is 62.5 µg/ml for dried rhizomes at ambient temperature or at the laboratory temperature. Nevertheless, for extracts from roots dried at the laboratory temperature or exposed to the sun, the minimum concentration of normalization was 62.5 µg/ml and 125 µg/ml respectively. This allows us to deduce that (i) the methanolic extracts of the rhizomes and the roots actually possess an antisickling activity and (ii) the active ingredients responsible for this antisickling activity are not photosensitive or the chemical groups of the responsible active ingredient the antisickling activity are not degraded in the presence of UV light.

Several studies reported that methanolic extracts of *C. longa* rhizomes contain 5-8% of curcuminoids, including curcumin that is attributed the responsibility for most pharmacological actions such as: anticarcinogenic, anti-inflammatory, cicatrizing, cholesterol-lowering , anti-inflammatory, antioxidant, antibacterial, anti-fungal, antivenom, antipyretic, analgesic, inhibits the action of HIV-1 integrase, and HIV-1 integrase protein replication, protects against the diabetic retinopathy and numerous other pathologies [1-10]. *C. longa* has a wide pharmacological potential and is a good candidate for the management of sickle cell disease.

Some authors have shown that curcuminoids prevent the oxidation of hemoglobin [5] while other authors reported that curcuminoids are degraded in the presence of UV light.

Sundayono [11] demonstrated that the pathways of phodegradation do not involve phenolic groups of curcuminoids. In view of our results, we believe that if the curcuminoids are responsible for the antisickling activity, these molecules are not totally degraded after their exposure to the UV light or the photodegradation does not affect the chemical group responsible for the antisickling activity. The antisickling activity of total methanolic extracts derived from rhizomes or roots of *C. longa* could also be due to other active ingredients, such as organic acids or anthocyanins. In fact, several studies of [13-20, 22] showed that the antisickling activity of plants is generally due to the presence of anthocyanins and organic acids. If we assume that the organic acids and anthocyanins of *C. longa* rhizomes and roots are responsible for the antisickling activity, then it can be assumed that these active ingredients are not photosensitive. However, it is would be appropriate to confirm this hypothesis by extracting these specific active compounds (curcuminoids, anthocyanins and organic acids) in order to evaluate their antisickling activity and/or to study their photodegradation.

4. CONCLUSION AND RECOMMENDATIONS

Methanolic extracts from the rhizomes and roots of *C. longa* possess the antisickling activity and photodegradation has no impact on the active ingredients of these extracts. Further studies are needed in order to identify the active ingredients responsible for this antisickling activity of the extracts from the roots and rhizomes of *C. Longa*, to extract the organic acids, anthocyanins and curcuminoids from C. longa rhizomes and roots for the evaluation of the antisickling activity, to assess the synergistic action of these different active compounds, to study their mechanisms of action and last to study the photodegradation or thermosensitivity of anthocyanins and organic acids in the roots and rhizomes of *C. longa*.

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