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In vitro Antisickling Activity of Anthocyanins from Ocimum canun (Lamiacea)

D.D. Tshilanda[1], D.V. Onyamboko[1], D. T. Mwanangombo[1], P.V. Tsalu[1], N.K. Misengabu[2], D.S.T. Tshibangu[1], K.N. Ngbolua[3], P.T. Mpiana[1*]
[1]Department of Chemistry, Faculty of Science, University of Kinshasa, P.O. Box 190 Kinshasa XI, Democratic Republic of the Congo
[2]Institut de Recherche en Sciences de la Santé IRSS, Kinshasa, Democratic Republic of the Congo
[3]Department of Biology, Faculty of Science, University of Kinshasa, P.O. Box 190 Kinshasa XI, Democratic Republic of the Congo.
*Corresponding author: Prof. Dr. PT Mpiana, Tel: (+243) 81 811 60 19 E-mail: ptmpiana@yahoo.fr
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ABSTRACT

Some medicinal plants used in Congolese traditional medicine in the management of sickle cell disease have showed interesting antisickling activity *in vitro*. In the present work, antisickling activity of different extracts from *Ocimum canum*, a medicinal and aromatic plant used in Congolese traditional medicine, was studied. Aqueous, methanolic, ethyl acetate, n-hexane and dichoromethane extracts were tested using Emmel's test. Polar extract (Aqueous, methanolic, ethyl acetate) showed antisickling activity when non polar (n-hexane and dichoromethane) were inactive. Anthocyanins extracts from *O. canun* leaves yielded 8.7 % and showed a strong antisickling activity. This biological activity is dose-dependent and reaches a sickle shape normalization rate of 88.5% at a minimal normalization concentration of 15 µg/ml.

Keyword: *Ocimum canum*, anthocyanins, antisickling activity, sickle cell disease, normalization rate.

INTRODUCTION:

Several diseases cause million deaths in the world, and particularly in Africa. Among these, is the sickle cell disease (SCD) or sickle cell anemia that affects more than 50 million people. Each year, nearly 100,000 children are born worldwide with this hemoglobinopathy [1-4].

SCD is a chronic disease due to replacement of a polar amino acid (glutamic acid) by a less polar one (valine) that causes a polymerization of hemoglobin S (HbS) in the red blood cells. This aggregation modifies the shape of blood cells and makes them fragile and less flexible; what causes many complications of sicklers [1, 5-7].

Several therapeutic options were tried in order to fight against SCD without appropriate solution for poor African population. All of these therapeutic approaches are either expensive or toxic and are not accessible to the populations with low incomes [1, 6, 8].

In fact, people in developing countries have resort to medicinal plants in order to treat several diseases among which SCD [9-16]. This could be proposed as an alternative solution to treat this disease that affects mainly African people and their migrants. Indeed, several studies showed that among plants quote in traditional medicine as being able to treat SS anemia, a good number showed an *in vitro* antisickling activity [16-22].

Our research team listed indeed, a number of plants used in traditional medicine against SCD in Democratic Republic of the Congo (RDC) and tested their antisickiling activity [17-19; 23-28]. These results showed that anthocyanins and organic acids are among active compounds [23-28].

Ocimum canun Sims also known as *Ocimum americium L.* is called African mint , American basil , Asian basil or hairy basil and is known for its essential oil and several therapeutic uses [29]. However, its antisickling activity have never been reported. In DRC, *O. canum* is an edible plant used for its aroma as two others species of *Ocimum* genus, *O. basilicum* and *O. gratissimum* and is called " Luenyi " in tshiluba (DRC) [29,30].

MATERIALS AND METHODS

Plant materials

Plant samples used in this works (leaves of *Ocimum canun*) were harvested in the vicinity of Mbuji-mayi city in the central part of RDC on May 2013. The collected materials were identified and deposited at herbarium of the Faculty of Science, University of Kinshasa.

Extraction

Aqueous, methanolic, ethyl acetate, n-hexane and dichoromethane extracts were obtained by maceration of 100g of dried powdered sequentially. Fraction resulting was filtered and the solvent was evaporated under reduced pressure using a rotary evaporator.

Extraction of anthocyanins was then done using 100g of dried powdered plant material with acidified methanol (1% HCl) and diethyl ether according to the universal procedure [23-28].

Biological material

The blood samples used for the bioassays in this study were taken from adolescent SCD patients attending the "Centre de Medicine Mixte d'Anemie SS" (CMMASS), located in Kinshasa, DRC. A written consent for each patient was approved by the national ethic committee (N° BE117). Ethical clearance on the use of SS blood was strictly observed according to international rules [31].

In order to confirm their SS nature, the above mentioned blood samples were first characterized by hemoglobin electrophoresis on cellulose acetate gel at pH 8.5 and then stored at 4°C in a refrigerator.

Biological assays

Emmel's tests were performed as previously reported [2-4, 18]. The red blood cells (RBCs) digitized micrographs were treated with a computer assisted image analysis system (Motic Images 2000, version 1.3), Statiscal analysis and curves were processed using Microcal Origin 8.6 package Software. All anti-sickling experiments were carried out in triplicate using a sodium citrate suspension of freshly collected blood [18, 21-25].

RESULT AND DISCUSSION

Extraction yields

Figure 1 shows the yield of extraction of *Ocimum canun* leaves.

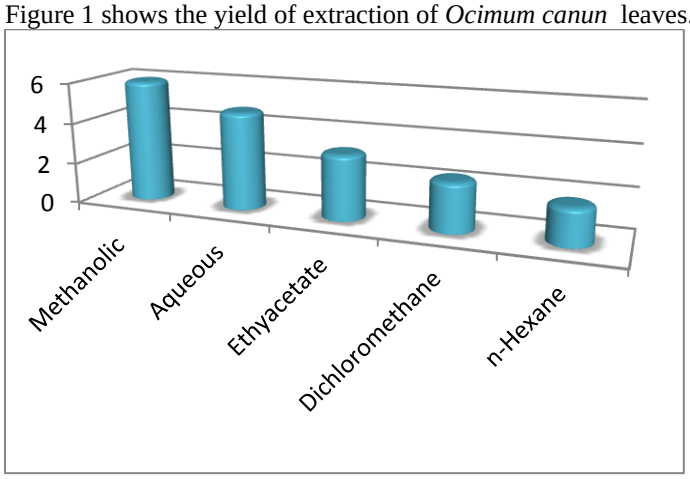


Figure 1: Extraction yields (%) of O. canun leaves

It can be noticed in this figure that the unpolar fractions have lower yields than polar ones. That indicates the majority of metabolites in the leaves of *Ocimun canun* are those which pass easily into the polar solvents [2,4, 6-9, 11].

Antisickling activity

Figures 2 , 3 and 4 show respectively micrographies of SS blood alone in a NaCl 0.9% solution (control) and the SS blood incubated with the aqueous total extract and acetate extract of *O. Canun*.



Figure 2: Morphology of erythrocytes of untreated SS blood (control) (X500), [NaCl 0.9% ; $Na_2S_2O_4$ 2%].

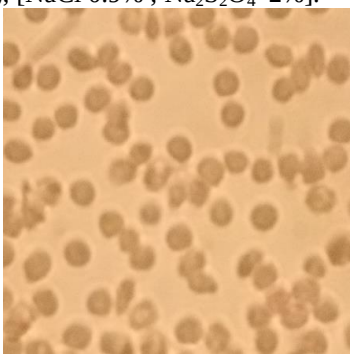


Figure 3: Morphology of erythrocytes of SS blood treated with aqueous extract of *O. canun* (X500), [NaCl 0.9% ; $Na_2S_2O_4$ 2%].

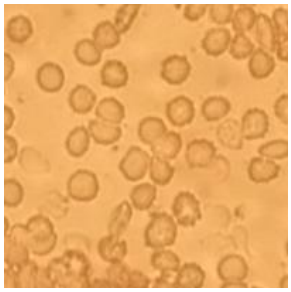


Figure 4: Morphology of drepanocytes treated with ethyl acetate extract (X500) [NaCl 0.9% ; $Na_2S_2O_4$ 2%].

As it can be seen from these micrographies, the control contains the majority of sickle-shaped erythrocytes, confirming the SS nature of the blood (Fig.2). When mixed with the leaves aqueous extract of *O. canun* (Fig. 3), the majority of erythrocytes are reversed normal-shape. This fact indicates that the aqueous extract has an antisickling activity. The ethyl acetate extract shows also (Fig.4) a good normalization effect of drepanocytes in hypoxic condition.

Figure 5 give the dose dependent antisickling activity of methanolic extract of *O.canum* leaves.

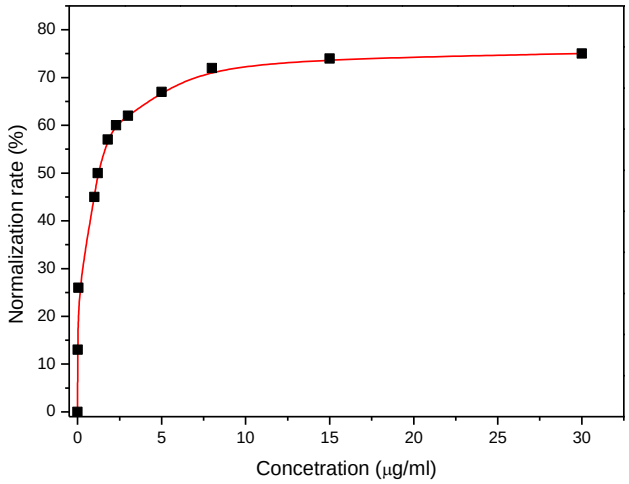


Figure 5: Evolution of the normalization rate of the drepanocytes form with the O.canum leaves methanolic extract.

Figure shows that the SS blood cell normalization rate increases with the methanolic extract dose until reaching the maximum threshold of which the normalization rate remains constant despite the increase of the extract concentration. The determined minimal concentration of normalization i.e. the weakest concentration of extracts for which the normalization rate maximum is 14 µg/ml for a maximal normalization rate of about 70%.

Table 1 summarizes the antisikling activity of different extracts.

Table 1: Antisickling activity of O.canum leaves extract

Extract	Antisickling activity
Aqueous	++
Methanolic	++
Ethyl acetate	+
Dichloramethane	-
n-Hexane	-

Legend:

++: high normalization rate (>50%);

+: low normalization rate (< 50%);

-: absence of normalization (< 10%).

As it can be noticed from the table 1, polar extracts show higher antisickling effect than none polar ones. The same behavior was already observed for other extracts from medicinal plants used in Congolese traditional medicine for management of SCD [2-8, 11, 19, 23-29]. This indicates that the secondary metabolites responsible of the antisikling effect have to be found in polar extracts. In fact, previous works showed that anthocyanins and organic acids and their derivatives are main phytochemical groups with antisickling activity [23-29, 31, 32]. So anthocyanins were extracted in order to test their antisickling activity.

Effect of anthocyanins extracts on sickle cell morphology

The extraction of anthocyanins from *O. canun* leaves yielded 8.7 %. This indicates that this plant exhibits a high content of anthocyanins compared to that of some others plants, among which another specie of *Ocimum*, *O. basilicum* that yielded 5.8 % [29].

Figure 6 shows micrography of the drepanocytes in the presence of anthocyanins extracted from *O. canun*

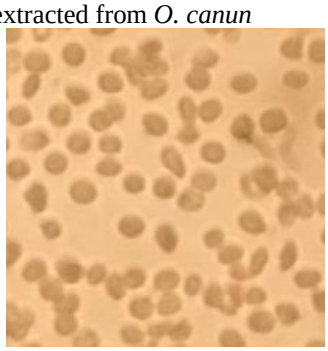


Figure 6: Morphology of drepanocytes treated with anthocyanins extract of O. canun (X500), [NaCl 0.9% ; Na2S2O4 2%,].

Compared to figure 2 (control), figure 6 shows that majority of sickle erythrocytes return to a circular normal shape indicating the antisickling activity of anthocyanin extract. This confirm the already observed effect for some others plants used in Congolese traditional medicine [2-8, 18, 19, 23-29]. In fact, anthocyanins could interact with HbS and compete with the polymerization reaction. This would inhibit the erythrocyte sickling process.

Figure 7 gives the evolution of the rates of normalization of the form of the drepanocytes with the concentration of anthocyanins extracted from *Ocimum canun* leaves.

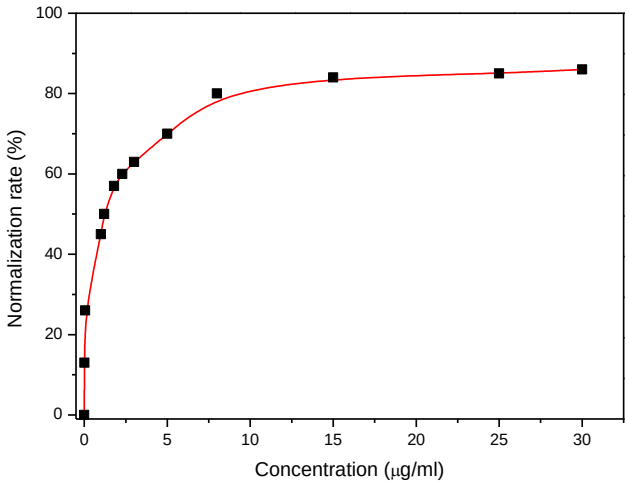


Figure 6: Evolution of the normalization rate of the drepanocytes form with the anthocyanins concentration.

This figure shows that normalization increases with the anthocyanins extract concentration and reaches a constant value at 15 µg/ml corresponding to a normalization rate of 88.5%. This high normalization rate justifies the use of *O.canum* leaves in traditional medicine to manage SCD.

CONCLUSION

The present study evaluated the *in vitro* antisickling activity of *Ocimum canun* Sims leaves extracts. Polar extracts showed higher biological than non-polar ones. This activity would be due to anthocyanins that showed a high normalization effect of drepanocytes. Fractioning of anthocyanin extract in other to determine active molecule and elucidate molecular structure is undergoing as well as antioxidant study of different extracts.

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