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Antisickling, antihemolytic and radical scavenging activities of essential oil from *Entandrophragma Cylindricum* (Sprague) Sprague (Meliaceae)

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ABSTRACT:
Essential oil of *Entandrophragma Cylindricum* from Masoko forest (Tshopo province, DR Congo) was extracted and tested for antisickling, antihemolytic and radical scavenging activities. The obtained results indicate that the extraction gives a yield of 0.85% of essential oil with a density of 0.8581 and refractive index of 1.5025 at 20°C. The essential oil showed a good antisickling activity comparatively to methanolic and aqueous extracts of the same plant with a maximal rate of red blood cells normalization of 89% and minimal concentration of normalization 80 µL/mL. When methanolic and aqueous have maximal rate of normalization of only 24 and 33 % respectively. The essential oil showed also the highest antihemolytic effect and a good radical scavenging effect.

Keyword: *Entandrophragma Cylindricum, antisickling, antihemolitic, radical scavenging activity, normalization rate*

INTRODUCTION:

Essential oils (EO) have long served as flavoring agents in foods and beverages, they are not only responsible of the pleasant odor of aromatic plants but also of biological activities of some aromatic and medicinal plants [1]. It was recently showed that some aromatic plants used in Congolese traditional medicine in the management of sickle cell disease possess antisickling activity *in vitro* [2-6].

Sickle cell disease (SCD) or sickle cell anemia (SCA) also called drepanocytosis, is a genetic blood disorder arising from a mutation in the ß-globin gene that leads to the replacement of glutamic acid residue by valine at the sixth position of the ß-chain of hemoglobin. This conduct to production of an abnormal hemoglobin, hemoglobin S (HbS). At low oxygen level (hypoxia), deoxy-HbS polymerizes inside the red blood cells (RBC). This formation of deoxy-HbS polymer makes the Hb insoluble, change the biconcave shape of the RBC and eventually cause cell lysis, which leads to the various clinical symptoms of SCD [7-12].

This chronic disease affects mainly sub-Saharan African. In this region, the carriers of sickle cell feature account for up to 20% with a prevalence between 25-30% in Central Africa. In Democratic Republic of the Congo (RDC) two per cent of the population i.e. about one and half millions of people are affected by this hemoglobin abnormality [9-18].

Some proposed therapeutic options seem inappropriate for low-income countries population, mainly in Africa. The World Health Organization (WHO) reported that 80% of the African population relies on herbal medicine for therapy. The extensive use of herbal medicine in Africa, has been argued to be linked to cultural and economic reasons. This is why the WHO encourages African member states to promote and integrate traditional medical practices in their health system [2, 15-20].

Indeed, phytotherapy appears to be a promising alternative therapy for SCD because a plant-based remedy will be more affordable for Africa because of it biodiversity richness. In DRC, our research team validated the antisickling activity of about 120 plants used in Congolese traditional medicine, including some aromatic plants [2-6, 9-21].

Recently, a ethnobotanical survey was conducted in Masako forest reserve, at Kisangani city (Thopo province) located in the north-east of DRC in order to collect, identify and determine botanical parameters of aromatic plants. Forty three aromatic plants species corresponding to eleven families and twenty nine genera were identified among which *Entandrophragma Cylindricum* (Sprague) Sprague [1].

Entandrophragma Cylindricum known as "Liboyo" in Kisagani (DRC) is generally called Sapeli or Sapele. It is a tree that can grow 40 - 60 meters tall. It's wood is very used but it is also known as medicinal plant . The bark decoction or maceration is taken to treat bronchitis, lung complaints, colds, oedema etc.[22].

This study aims to extract and determine antisickling, antihemolytic and radical scavenging activity of Essential oil of *E. Cylindricum.*

MATERIAL AND METHODS

2.1. Plant material

The used plant material was stem barks of *Entandrophragma Cylindricum* from Masako forest reserve in Tshopo province, Democratic Republic of the Congo. The identification of the plant was carried out by comparison with vouchers referenced at the herbarium of the Faculty of Sciences, University of Kisangani. Voucher specimen is on deposit at the same herbarium.

2.2. Essential oil distillatio

Essential oil has been produced by hydro-distillation as earlier reported [6]. A weighed amount of stem barks was immersed in a 500 mL round bottom flask of water and hydro-distilled. Water and essence were recovered in a decant bowl, and anhydrous magnesium sulfate was used for drying trace of water. Oil was stored in a dark glass bottle at 4°C.

2.3. Radical scavenging Activity

Radical scavenging activity was determined according to 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method as previously reported [5,17-19]. About 3.5 mL of 0.3 mmol/L solution of DPPH radical in methanol were added to either 0.5 mL solution of essential oil or to 0.5 mL of crude extracts solutions. Bioactive essential oil and extracts solutions were used at the same values of concentration in methanol for comparison. Each mixture was submitted to spectrophotometry (HITACHI U 5100 UV-vis Spectrophotometer) analysis. Mixture of essential oil or crude extracts solution with DPPH radical solution in methanol were shaken vigorously and

absorbances were recorded at 517 nm during 35 min as equilibrium time. DPPH radical scavenging has been determined by percentage of reduction that provides IC50 (concentration of essential oil or extract or ascorbic acid that reduces 50% of DPPH radical concentration) by extrapolation as antioxidant effectiveness. Ascorbic acid was tested as standard for comparisons.

Percentages of reduction were calculated according to the following equation:

$$I\% = \frac{A_{blanck} - A_{sample}}{A_{blanck}} x100$$

where A_{blank} is absorbance of blank and A_{sample} is absorbance of the tested sample.

2.4. Antisickling and hemolytic experiments

Blood samples used to evaluate the antisickling activity of the plant extracts were taken from known sickle cell anemia patients attending the pediatric unit of " Hopital de reference de Kabondo" located in Kisangani, DRC. None of the patients had been transfused recently with Hb AA blood. All antisickling experiments were carried out with freshly collected blood. In order to confirm their sickle cells nature, the above-mentioned blood samples were first characterized by hemoglobin electrophoresis on cellulose acetate gel, and then stored at ± 4°C in a refrigerator.

Emmel test

Blood sample was put in contact with plant extracts at different concentrations (with the physiologic solution as the dilution solvent) according to Emmel's test procedure as previously reported [12-15].

Hemolysis test

RBC were washed twice in physiological saline (NaCl 0.9 %, 1:5 v/v) by centrifugation at 3000 rpm for 10 min, re-suspended in phosphate buffer (150 mM, pH 7.4) containing 2 % sodium metabisulfite and incubated in the absence (control) or presence of essential oil (50 µg/mL of NaCl 0.9 %) at 37° C for 60 min. At fixed time points, aliquots of the blood samples were removed and centrifuged at 4000 rpm at ambient temperature for 5 min. The absorbance of the supernatant was measured at 540 nm and was expressed as the degree of hemolysis [9,10]

The rate of hemolysis inhibition (% HI) versus time was calculated from the absorbance of sickle erythrocyte (SS RBC) suspension by the relation:

$$HI(\%) = \frac{A_o - A_t}{A_0} x100$$

With A_0 as absorbance of untreated SS RBC suspension and A_t, the absorbance of treated SS RBC suspension.

2.5 Statistical analysis

The results are given as Mean ±Standard Deviation obtained from three independent experiments. The Microcal Origin version 8.5 Pro package software was used to treat the data. All data were analyzed at a 95% confidence interval (α= 0.05).

RESULTS AND DISCUSSION

3.1. Essential oil yield and physico-chemical properties

Hydro-distillation of 100 g of stem barks produced a total of 0.85 g (0.85%) of EO, relatively to the initial amount of fresh herbs used. The obtained EO had a density of 0.8581 and refractive index of 1.5025 at 20°C. It was clear and had good aroma. These results indicates that *Entandrophragma Cylindricum* gives a fairly high EO yield and a high refractive index compared to many of the aromatic plants [5]. So it can be exploited commercially.

3.2. Antisickling activity

Micrographies of SS blood in presence of EO of *E. Cylindricum* were compared to that of the same blood alone in a NaCl 0.9% solution used a negative control. The obtained results showed that majority of RBC are reversed normal-shape or normalized. In order to evaluate the antisickling activity of EO of *E. Cylindricum* and compare it to that of other drugs, it is necessary to determine the maximal rate of normalization (MRN), the minimal concentration of normalization (MCN) or the concentration that normalizes 50% of drepanocytes (ED50). Figures 1, 2 and 3 give the evolution RBC normalization rate with the drug concentration for respectively EO, methanolic and aqueous extracts of *E. Cylindricum*.

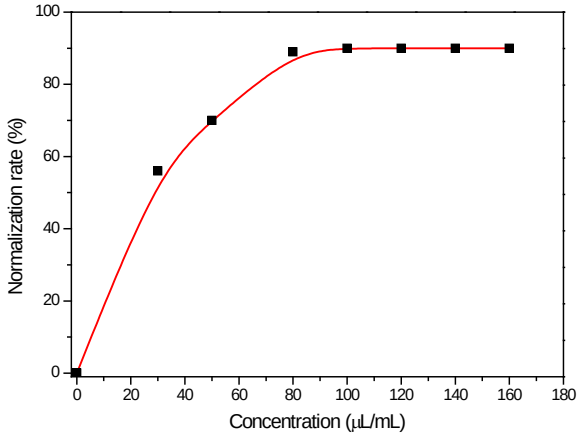

Figure 1: Evolution of the normalization rate of the drepanocytes form with the concentration of EO from *E. Cylindricum*

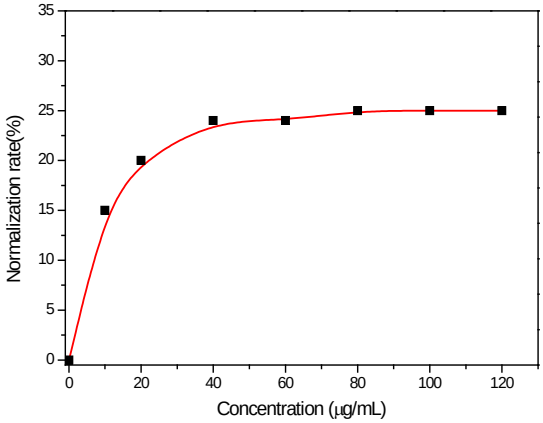

Figure 2 : Evolution of the normalization rate of the drepanocytes form with the concentration of methanolic extracts of *E. Cylindricum*

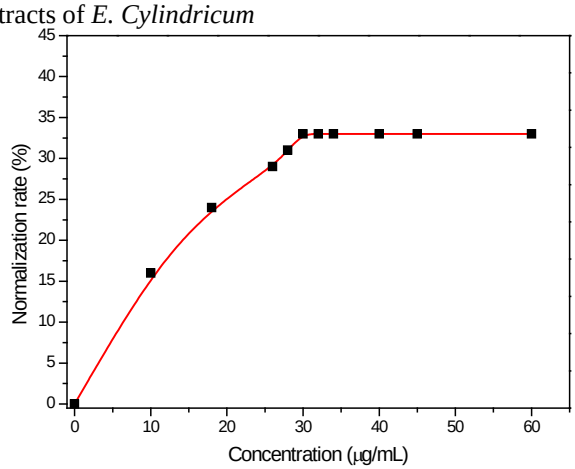

Figure 3: Evolution of the normalization rate of the drepanocytes form with the concentration of aqueous extracts of *E. Cylindricum*

These figures show that the drepanocytes normalization rate increases with the concentration of different extracts until reaching the maximum threshold of which the normalization rate remains constant despite the increase of the extracts concentration. This indicate that the sickle cells normalization rate is dose-dependent as already shown in our previous works

[3-5, 12-21]. The Maximal rate of normalization, the weakest concentration of extracts for which the normalization rate is maximum or minimal concentration of normalization (MCN) and the concentration that normalizes 50% of drepanocytes (ED50) of EO, methanolic and aqueous extracts are given in table I.

Table I: Maximal rate of normalization, minimal concentration of normalization and ED50 of different extracts

Extract	MRN (%)	MCN	ED50
EO	89	80 µL/mL	40µL/mL
ME	24	40µg/mL	-
AE	33	30µg/mL	-

Legend: EO: essential oil; ME: methanolic extract; AE: aqueous extract; MRN: maximal rate of normalization; MCN: minimal concentration of normalization; ED50: concentration that normalizes 50% of drepanocytes

It can be seen from this table that only the EO shows high normalization rate that can rich 89% so ED50 could be calculated. For methanolic and aqueous extracts the maximal normalization rate is under 50%, ED50 could not be determined. This result indicates that the antisickling activity of *E. cylindricum* is mainly due to its essential oil. Tshilanda et al [4,5] have recently shown that EO of some *ocimum* species has high antisickling activity.

3.3. Antihemolytic activity

The effect of drugs on RBC membrane damage can be evaluated by comparing % of hemolysis or inhibition of hemolysis of untreated and treated red blood cells in isotonic condition (0.9% NaCl)

Figure 4 shows the evolution of the inhibition rate of hemolysis of sickle cells in the presence EO, methanolic and aqueous extract of of *E. cylindricum.*

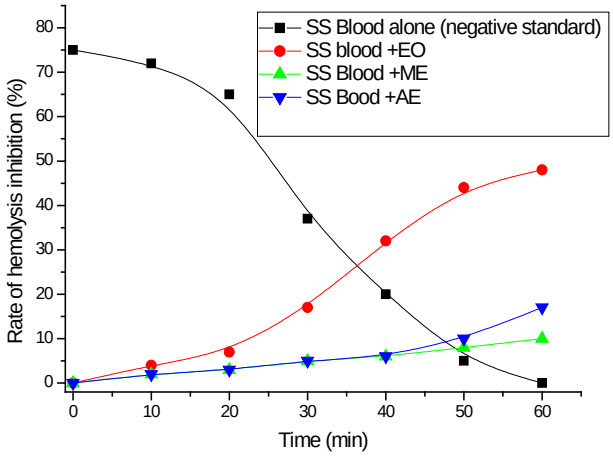

Figure 4: Evolution of the inhibition rate of hemolysis of sickle cells in the presence EO, methanolic and aqueous extract of *E. cylindricum*

As it can be seen from this figure, the hemolysis inhibition rate of sickle RBC alone decreases with time but in presence of essential oil, methanolic and aqueous extracts the inhibition rate increases with time. This indicates that these extract have an antihemolytic effect on sickle RBC. Indeed, the sickling modifies the membrane flexibility, which would make it more fragile and would increase the precocious risk of hemolysis. The presence of *E. cylindricum* extracts could stabilize the RBC membrane and prevent them to hemolysis.

As it can be saw in the figure, the EO shows a higher membrane protective effect than methanolic and aqueous extracts. This effect was also found for anthocyanin extracts from some medicinal plants used in management of sickle cell disease in Congolese traditional medicine [21]. This effect was explained by the anthocyanins antioxidant or free radical scavenging activity, that could prevent hemoglobin from oxidizing in methemoglobin (MetHb) and inhibit the generation of free radicals. It was also stated that the anthocyanin extracts would exert this protective effect according to their reducing properties preventing that the lipids membrane, hemoglobin and the enzymatic equipment are destroyed or inactivated by oxidation [9,10, 18,19].

So it is important to evaluate the antioxidant activity of theses extracts. Indeed, a apart from the inhibition of hemoglobin S polymerization, endothelial injury and the erythrocyte membrane, free radicals production have been defined as new target in sickle cell disease therapy [7,21]. Because of the reduced glucose methabolism and the low activity of both the glutathione reductase system and methemoglobin reductase which are involved in the protection of Hb and membrane from oxidative breakdown, MetHb, a biomarker of oxidative stress and radical oxygen species (ROS) are build up spontaneously in drepanocytes. The ROS would act as biological nucleophile in the de-esterification of membrane lipids leading into hyperhemolysis of drepanocytes [21].

3.4. Antioxydant activity of essential oil

Percentages of reduction of the DPPH radical vs time at different concentrations by essential oil are shown in figure 5.

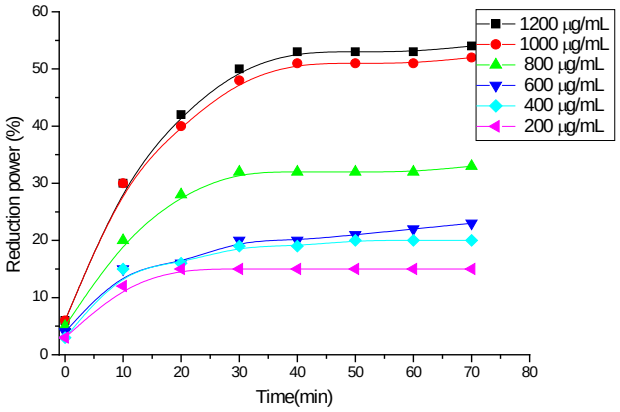

Figure 5: Evolution of DPPH Reduction power of EO with time at different concentrations.

This figure shows that the reduction power of EO from *E. cylindricum* on DPPH radical increases with time and reach a maximum at about 30min. The reduction power also increases when the concentration of EO increases. This indicates that EO from *E. cylindricum* has a radical scavenging activity. But calculated IC50 give a high value of 1052 µg/mL or 1.052 mg/mL compared to that of ascorbic acid used as positive standard (7.56 µg/mL). But this value is lower that of EO from *Ocimum basilicum* (1.18mg/mL) [6].

CONCLUSION

The results of this study suggests that essential oil from *E. cylindricum* not only has antisickling activity but it also inhibits hemolysis of sickle cells probably by scavenging free radicals produced spontaneously within the sickle red blood cells. Determination of the composition of this essential oil is under study.

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