

Antihemolytic, radical scavenging and antibacterial activities of essential oil of *Fagara macrophylla* (Oliv) Engl from Masako forest reserve (RD Congo)

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Abstract: Essential oil of *Fagara macrophylla* from DR Congo was tested for antihemolytic, radical scavenging and antibacterial activities. The extraction gives an essential oil yield of 1.08 %, density of 0.7066 and refractive index of 1.4721 20°C. Aqueous and methanolic extracts showed high antisickling activity with the normalization rate (NR), the minimal concentration of normalization (MCN) and the concentration that normalized 50% of RBC (ED50) for aqueous extract of respectively 93%, 8µg/mL and 0.43µg/mL and for the methanolic extract NR, MCN and ED50 are respectively of 91%, 12µg/mL and 0.60µg/mL. The essential oil showed no antisickling activity but weak antihemolytic and radical scavenging activities. The *F macrophylla* essential oil showed higher bacterial activity on *Staphylococcus aureus* than the two antibiotics used as standards (Meropenem, Ceftriaxone).

Keywords: *Fagara macrophylla*, DR Congo, Masako, antihemolytic, antisickling, antibacterial.

INTRODUCTION

The Democratic Republic of Congo (DRC) is known for the extraordinary richness of its flora. The forests cover approximately 62% of its territory. This is the second largest block of tropical forest in the world and boasts a wide variety of medicinal plant species [1]. Medicinal plants are the key product for the Congolese population. Almost all Congolese populations, both urban and rural, depend on medicinal plants for their health care needs because the costs of conventional drugs are often unaffordable [2,3].

Sickle Cell Disease (SCD) or sickle cell anemia is one of the illnesses for which the African population relies on medicinal plants for their treatment. Sickle cell anemia is a genetic blood disorder which is widespread all over the world, with an important affection in Africa and particularly in sub-Saharan regions [4-12]. This chronic disease is caused by abnormal hemoglobin named hemoglobin S (HbS). HbS comes from a mutation at the β^6 position that led to the substitution of glutamic acid a polar amino acid, by valine a non-polar amino acid. This structural modification influences significantly physical and chemical properties of hemoglobin and decreases the affinity of hemoglobin for oxygen [4,5].

At hypoxic condition, HbS polymerizes inside the erythrocytes into a gel or further into fibers leading to a drastic decrease in the erythrocytes deformability. Polymerization and precipitation of this abnormal hemoglobin within the red cells cause the change of their shape from their normal globular form into one resembling a sickle. This shape modification induces vaso-occlusive crises, chronic hemolytic anemia and other sicklers' problems [4-13].

Unfortunately, current proposed therapies are very limited and even not efficient. These include bone marrow transplantation, repeated blood transfusion, the use of hydroxyurea etc. Not only these treatments were ineffective, they are also expensive for the poor African population and may present HIV / AIDS infection risk [4-7].

An alternative strategy of the management of the sickle cell anemia is the use of bioactive medicinal plants, the identification of the novel ant sickling molecules plants and the use of nutraceutical [13-22].

In DRC, our research team has initiated a large ethno pharmacological survey in order to identify plants that are used in the management of sickle cell anemia and to verify the effectiveness thereof. About 120 medicinal plants were identified among which *Fagara macrophylla*. Fewer molecules were isolated from some Congolese medicinal plants and a phytomedicine made of three most efficient edible plants is under experimentation [2,3,6-12,14-22]. Recently, essential

oil (EO) from *Ocimum* species has shown antisickling activity [23-27].

Fagara macrophylla (Oliv) Engl (or *Zanthoxylum macrophylla*) synonym of *Zanthoxylum gillettii* (De Wild.) P.G.Waterman [28, 29] is of the same genus than *Zanthoxylum xanthoxyloides*, one of the first plants to be tested *in vitro* for its antisickling activity. It is known for its different uses in traditional medicine and some molecules were isolated from it [29-31]. But not enough is known on its essential oil.

The aim of this work is to test the antisickling effect of *Fagara macrophylla* (Oliv) Engl essential oil. But as oxidant stress is implicated in the Red Blood Cell (RBC) physiopathology and due to the sensibility of sicklers to some infections, radical scavenging and antibacterial activity of *F. macrophylla* is also tested.

MATERIAL ET METHODS

Plant material

The used plant material was stem barks of *Fagara macrophylla* (Oliv) Engl 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.

Essential oil distillation

Essential oil has been produced by hydro-distillation as earlier reported [26]. 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.

Radical scavenging Activity

Radical scavenging activity was determined according to 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) method as previously reported [26, 32]. 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:

$$I \% = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100$$

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

Antisickling

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 $\pm 4^{\circ}\text{C}$ 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 [6-22].

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 $\mu\text{g}/\text{mL}$ 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 [6,7].

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

$$HI (\%) = \frac{A_o - A_t}{A_o} \times 100$$

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

Antibacterial activity

Microbial strains (*E.coli*; *salmonella spp.*; *pseudomonas aeruginosa*; *streptococcus D* and *staphylococcus aureus*) were obtained at the Biotechnology Laboratory of Kisangani University. Antimicrobial activity was tested using disk-diffusion method as previously described [33].

Statistical Analysis

The results are given as Mean \pm Standard Deviation obtained from three independent experiments. The Microcal Origin version 8.5 Pro package software was used to treat the data.

RESULTS AND DISCUSSION

Results

Essential oil yield and physico-chemical properties

Hydro-distillation of stem barks of *F. macrophylla* gives a yield of EO of 1.08 %. The

essential oil has a good aroma, white color and clear appearance. The determined density and refractive were 0.7066 and 1.4721 respectively at 20°C.

Antisickling and antihemolytic activities

Figures 1 and 2 give the evolution RBC normalization rate with the drug concentration for respectively aqueous and methanolic extracts of *F. macrophylla*. It can noticed that Essential oil from stem bark of *F. macrophylla* showed no effect on erythrocytes form at all used concentration

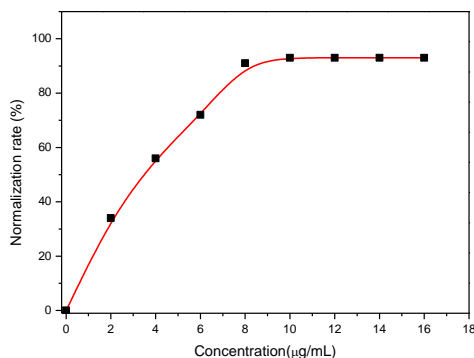


Fig-1: Evolution of the normalization rate of the sickle red cell form with the concentration of aqueous extract from *F. macrophylla*

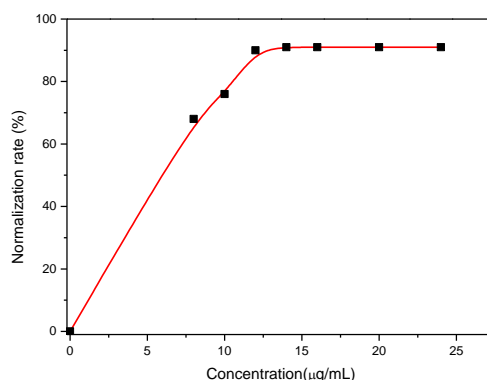


Fig-2: Evolution of the normalization rate of sickle red cells form with the concentration of methanolic extracts of *F. macrophylla*

These figures show that the drepanocytes normalization rate increases with the concentration of aqueous and methanolic extracts until reaching the maximum threshold of which the normalization rate remains constant despite the increase of the extracts concentration.

The normalization rate (NR), the minimal concentration of normalization (MCN) and the concentration that normalized 50% of RBC (ED50) for

aqueous extract are respectively 93%, 8µg/mL and 0.43µg/mL. For the methanolic extract NR, MCN and ED50 are respectively of 91%, 12µg/mL and 0.60µg/mL.

Figure 3 gives the evolution of the inhibition rate of hemolysis of sickle cells in the presence of essential oil (EO), aqueous (AE) and methanolic (ME) extract of *F. macrophylla*.

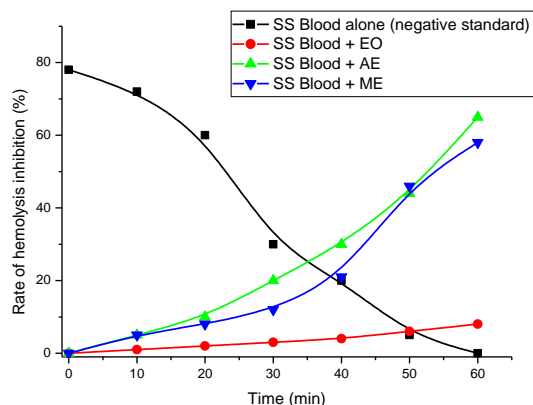


Fig-3: Evolution of the inhibition rate of hemolysis of sickle cells in the presence essential oil (EO), aqueous (AE) and methanolic (I) extract of *F. macrophylla*.

This figure shows that in the absence of extracts, the hemolysis inhibition rate of sickle red blood cells decreases with time but in presence of essential oil, methanolic and aqueous extracts the inhibition rate increases with time. But this increase is very weak for the *F. macrophylla* essential oil.

Antioxydant activity of essential oil

Percentages of reduction of the DPPH radical vs time at different concentrations by essential oil are shown in figure 4.

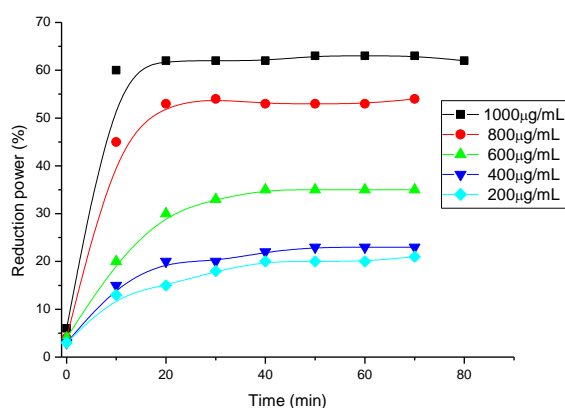


Fig-4: Evolution of DPPH Reduction power of EO with time at different concentrations

As it can be noticed from this figure the reduction power of essential oil from *F. macrophylla* on DPPH radical increases with time and reach a maximum after 20 min. The reduction power also increases when the concentration of EO increases. The calculated IC50 gives a value of 786±24 µg/mL.

Antibacterial activity

Table 1 gives the halo disc diameter values obtained on different microbial strains in the presence of EO of *F. macrophylla*.

Table-1: Effect of EO on different microbial strains

Microbial strains	Category	Diameter (cm)		
		Essential oil	Antibiotics	
			Meropenem	Ceftriaxone
<i>E. coli</i>	gram -	12	22	20
<i>Salmonella spp</i>	gram -	7	26	18
<i>Pseudomonas aeruginosa</i>	gram -	10	17	10
<i>Streptococcus D</i>	gram +	13	17	5
<i>Staphylococcus aureus</i>	gram +	15	11	2

Results from this table show that for *Staphylococcus aureus* the *F macrophylla* EO is more effective than the both antibiotics used as positive standard. For *Streptococcus D F macrophylla* EO is more effective than one of antibiotics (Ceftriaxone). Essential oil from *F macrophylla* is as effective as Ceftriaxone on *Pseudomonas aeruginosa* strains. But for *E. coli* and *Salmonella spp* the EO showed less effect than the two used antibiotics.

DISCUSSION

The obtained results indicate that *F macrophylla* gives a fairly high EO yield and a high refractive index compared to many of the aromatic plants [26]. With its good aroma this EO can be exploited commercially.

F. macrophylla is known for its antisickling activity [11, 12, 30]. This is confirmed by high normalization obtained in this work for its aqueous and methanolic extracts and its low MCN and ED50 for this two extract. Indeed, the obtained values show that this plant is one of the most active plants used in Congolese traditional medicine [11,12]. The high activity of the aqueous and methanolic extracts indicated that the antisickling activity of this plant is due to polar molecules. In fact, it was shown that the activity of this plant is due to an organic acid [24, 25, 27, 30]. The EO from *F. macrophylla* showed no antisickling activity but has a low antihemolytic activity compared to that of aqueous and methanolic extracts (Fig. 3). This indicates that even if the *F. macrophylla* do not contribute to antisickling effect of the plant like EO from *Ocimum* species [24-27] it could contribute to the RBC membrane stabilization effect of *F. macrophylla*.

Indeed, the sickling modifies the membrane flexibility, which would make it more fragile and would increase the precocious risk of hemolysis. The presence of *F. macrophylla* EO could contribute to the stabilization of the RBC membrane and prevent them to hemolysis.

Obtained results indicate that EO from *F. macrophylla* has a radical scavenging activity (Fig.4). But calculated IC50 give a high value of 786 µg/mL compared to that of ascorbic acid used as positive standard (7.56 µg/mL). But this value is lower than that of EO from *Ocimum basilicum* (118 µg/mL).

It is important to evaluate the antioxidant activity of EO. Indeed, 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 [26,32].

Because of the reduced glucose metabolism 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 built up spontaneously in drepanocytes. The ROS would act as biological nucleophile in the de-esterification of membrane lipids leading into hyperhemolysis of drepanocytes [32].

Essential Oil of *F. macrophylla* showed notable antibacterial activity compared to standard antibiotics mainly on gram+ strains. This confirms the results of Oyediji *et al.* [29]. In fact, antibacterial activity of plants used in the management of sickle cell disease is with great interest since infection is a significant contributor to morbidity and mortality in SCD. The sickle gene confers an increased susceptibility to infection, especially to certain bacterial pathogens, and at the same time infection provokes a cascade of SCD-specific pathophysiological changes. Historically, infection is a major cause of mortality in SCD, particularly in children, and it was implicated in 20–50% of deaths in prospective cohort studies over the last 20 years. Worldwide, it remains the leading cause of death, particularly in less developed nations [13].

CONCLUSION

The obtained results indicate that the known antisickling activity of *F. macrophylla* is due to polar molecules contained in aqueous and metabolic extracts. Essential oil does not show antisickling activity but possesses weak antihemolytic and radical scavenging activities. Essential oil also showed a significant antimicrobial activity. Determination of the composition of this essential oil is under study.

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