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*In vitro*antisicklingactivity of the aqueous extract of a combination of three plants: *Jatropha grossypiifolia, Justicia secunda* and *Parquetina nigrescens* from East Côte d'Ivoire.

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Abstract: *Sickle cell disease results in hemolytic anemia, vaso-occlusive crisis and susceptibility to infections. In Côte d'Ivoire, its prevalence is estimated at more than 14%. Patients use traditional pharmacopoeia with medicinal plants to treat themselves. The objective of this study was to verify the effectiveness of the aqueous extract of the composition of three medicinal plants (**Jatropha grossypiifolia**, **Parquetinanigrescens and Justicia secunda**) used in the treatment of sickle cell disease at East of the Côte d'Ivoire.*
The Emmel method taken up by Imaga was used to study the reversibility of sickle cells, as a function of the incubation time by the aqueous extract relative to the controls (NaCl and phenylalanine) on blood samples from SS homozygous patients. Concentrations of 10, 5, 0.5 and 0.05 mg / mL of the aqueous extract were contacted with sickle cells after pretreatment sickling cells with 2% sodium met bisulfite solution. Sickle cell determination was performed every 30 minutes for 120 minutes using a SQA Vision microscope. The extract showed activity on sickle cell reversion. The analysis of the histogram shows a decreasing rate according to the positive control Phenylalanine. This activity is dose-dependent (65, 70, 75 and 80%) vs Concentrations of 0.05;0.5;5 and 10 mg /mL. The reversion rate is 80% for the aqueous extract. Phytochemical screening showa correlate between the phenolic compounds with the antisickling activity of the aqueous extract plant combination. The use of the aqueousextract threeplant combination in a traditional environment would be justifiedin East Côte d'Ivoire.
Key words:*Antisickling activity, aqueous extractcombination, Jatropha grossypiifolia, Justicia secunda and Parquetina nigrescens, Ivory Coast.*

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I. Introduction

Sickle cell disease, or sickle cell anemia, is a genetic disorder with autosomal recessive inheritance. It is hemoglobin disease due to a point mutation on the gene coding for the beta chain of hemoglobin. This mutation induces the synthesis of an abnormal hemoglobin called hemoglobin S (HbS). In hypoxia, we are witnessing the polymerization of HbS. This promotes the deformation of red blood cells that take the shape of a sickle. In the homozygous individual, the disease results in hemolytic anemia, which is often accompanied by painful crisis and increased susceptibility to infections[1].

Migrations, consanguineous marriages and marriages with unknown hemoglobin status are contributing to the spread of this disease throughout the world. According to WHO, nearly 120 million people are affected worldwide. InCôte d'Ivoire, the prevalence of this disease is reflected in a prevalence rate of more than 14% of the total population of which more than 2% are severe forms[2].Several treatment modalities have been proposed to relieve patients, including bone marrow transplantation, genetic therapy, repeated blood transfusions, and hydroxyurea[3]. But only bone marrow transplantation provides satisfactory corrections to sickle cell patients[4]. Unfortunately, this highly specialized and expensive treatment approach is not available for most low-income patients. Its seriousness and its socio-economic repercussions make sickle cell disease a public health problem in the countries of Black Africa.According to the World Health Organization (WHO), more than 80% of the world's population uses traditional medicine to treatto deal with his health problems[5]. This crazeis explained by the fact that many diseases are treated satisfactorily and cheaply by plants[6].Usingto traditional medicine is also favored by the low purchasing power of the target populations. In order to make sure that medicinal plants are used, several plant extracts used in a traditional environment have been evaluated for their antifungal activity in some African countries[7, 8 ;9].This study is part of this same line. But it aims to make a contribution to the fight against sickle-cell anemias by searching for active molecules from the Ivorian flora. However, plant anti-sickle cell activity and toxicity have been poorly studied in Côte d'Ivoire[9]. The objective of this study is to identify the different chemical groups of *Jatropha grossypiifolia, Parquetina nigrescens* and Justicia secunda and to verify in vitro, the antisicklingactivity of the aqueous extract of the combination of three plants.

II. Material And Methods

This is an experimental study that took place in Pasteur Institute of Côte d'Ivoire(IPCI) to the Biology of Immunity Pole in Abidjan. The extraction was carried out at the Biosciences Training and Research Unit (UFR) at the Laboratory of Pharmacodynamics-Biochemistry, the Phytochemical Screening was done at UFR of the Pharmaceutical and Biological Sciences in the Pharmacognosy Laboratory, the samples were taken at the Yopougon University Hospital in the Clinical Hematology Department and then sent for the evaluation of antisickling activity

Plant material

The leaves of *Jatropha grossypiifolia, Justicia secunda* and *Parquetinanigrescens* were harvested from Abengourou in East of Côte d'Ivoire. Plant identification was done at the National Floristic Center of Félix Houphouët Boigny University. These leaves were washed, cut and then dried at a temperature of 25 to 30 ° C, onthislaboratory. After three weeks of drying, they were crushed and powdered separately using a Severin brand crusher. The powders obtained were put together in the proportions of 33.34 g each threeplant to form a total composition of 100 g.

Preparation of extracts

The preparation of the total aqueous extract was carried out according to the method of Zirihi[10]. One hundred grams (100g) of vegetable powder were dissolved in one liter of distilled water. The mixture was then homogenized 10 times for 2 minutes / ppm using a Severin ® brand blender. The homogenate obtained was drained in a square of fabric and then filtered successively three times on hydrophilic cotton and spun again on wahtman paper (3 mm). The filtrate was evaporated at 55 ° C. using a venticell®. The powder obtained is the total aqueous extract. The different concentrations (0.05, 0.5, 5 mg / ml) were prepared from these stock solutions for carrying out the antisickling activity.

Human material

The human material was composed of SS red blood cells from two patients whose hemoglobin status was proven by the method of electrophoresis of hemoglobin at the Yopougon University Hospital in the Clinical Hematology Department.

Inclusion criteria

To be included in this study, the blood should come from homozygous sickle cell patients who have not undergone blood transfusions for the two months prior to the blood test, regardless of age and gender.

Collection and conditioning of blood samples

For this study we obtained the agreement of all the ethic comity and informed consent approved by patients wishing to participate in the study. Venous blood sampling of each SSFA2 major homozygous patients was collected in a labeled violet tube containing ethylene diamine tetracetat (EDTA). These samples were placed in a cooler containing cold accumulators and then conveyed at 4 ° C to IPCI**.**

Methods

Phytochemical Screening

Phytochemical screening is a qualitative test, to confirmthe presence or absence of chemical compounds in a plant product. According to the method of Nemlin and Brunel[11]:

- Sterols and polyterpenes were searched for by Liebermann's reaction.
-The reaction with ferric chloride (FeCl3) characterize the polyphenols.
- Flavonoids were sought by the cyanidine reaction.
-The search for tannins was made from the reagent Stiasny.
- Quinone substances were searched for from the Bornstraëgen reagent.
- The saponosides were determined by the foam index.

Antisickling activity

In vitro screening of the herbal extracts for antisickling properties with blood samples collected from confirmed non-crisis sickle cell individuals was carried out using methods by Imaga[12]. Blood samples were

washed Tris in 0.15 M saline phosphate buffer, pH 7.4 to obtain the red cells which were then re-suspended in normal saline for the analysis. The blood cell suspensions were then pre-incubated with 0.05; 0.5; 5: 10 mg/ml concentrations of the crude aqueous extracts in the presence of 2% Sodium met bisulfite solution.Then, microscopically analyzed for the time course (120 min) for sickling of erythrocytes and percentage inhibition sickling compared with Phenylalanine as control. A plot of percentage sickling inhibition against extract concentration was analyzed for possible explanation of the observed antisickling effect.
The reversibility rate of the sickle cells is determined in each field of the SQA Vision microscope. Phenylalanine was used as a positive control and NaCl as a negative control. The data analyzes were performed using the EXCEL 2013 software.

III. Result

Plant yield

Figure 1 gives the yield of each three plants and the combination. This figure shows that *Justicia secunda* gives a good yield compared to the others while the combination gives an average yield of the three.This yieldwas 14.25%

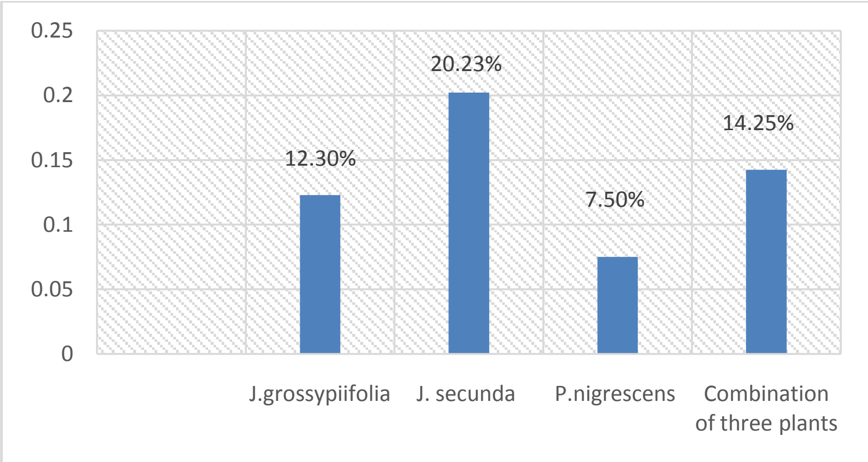

Figure n ° 1: Histogram of the different yield plants and the combination of three plants

PhytochemicalScreening

Phytochemical analysis of the combination of the three plants revealed the presence of the major chemical groups: polyphenols, alkaloids, terpenoids and heterosids (Table I). Gallic tannins are in trace form in the combination of the three plants, and no exist on two of them.

Table I: Chemical compounds present in plants

FAMILY	Plants / Chemical composition aqueous extract	***Justicia secunda*** Leaves	***Parquetina nigrescens***Leaves	**Jatropha grossypiifolia** Leaves	Combination of plants
TERPENOIDS	Sterol / Polyterpene	ST^+	ST^+	ST^+	ST^+
POLYPHENOLS	Polyphenols	Pol^+	Pol^+	Pol^+	Pol^+
	FLAvonoïds	Fla^+	Fla^+	Fla^+	Fla^+
	Catechetical Tannins	$T\,C^-$	$T\,C^+$	$T\,C^-$	$T\,C^+$
	Gallic Tannins	$T\,G^-$	$T\,G^+$	$T\,G^-$	$T\,G^{-/+}$
ALKALOIDS	QuinonesSubstances	$S\,Q^+$	$S\,Q^+$	SQ^+	SQ^+
	Alkaloids	ALC^+	ALC^+	ALC^+	ALC^+
HETEROSID	Saponin	SAP^+	SAP^+	SAP^+	SAP^+

(+) presence; (-) absence; (+ / -) trace
ALC = Alkaloids; T C = Catecheticaltannins; TG = Gallic tannins, FLA + = FLAvonoïds; POL + = polyphenols; ST = Sterols and Terpenes; SQ = Quinones; SAP = SAPonin.

Antisickling activity

Figures 2 to 5 show the results of anti-sickle cell activity of the aqueous extract for the combination of three plants at different concentrations on the blood of patients.

These results show the anti-sickle activity of the aqueous extract of the combination of plants on blood samples of SS individuals previously treated with sodium met bisulfite in an oxygen-free medium.The percentage of sickle cell decreased over time, especially it was accentuated between 0 and 30 min for all concentrations of the extract, compared to the negative control containing more than 80% of sickle cells.

In fact, the aqueous extract applied to the blood of the patients gives a sickling reversion rate of 65%, 69%, 73% and 80% according to the concentrations of 0.05, 0.5, 5 and 10 mg / ml respectively. The highest dose having most effective antisicklingactivity than the other concentrations. We also find a dose-dependent effect for the plant combination, as the lower the concentration, the higher the sickle cell count.

Between 30 and 120 min the reversion is progressive but slow. This phenomenon is as well observed for the extract as the positive control (phenylalanine). After 120 minutes, this drop was 20% compared to 80% at the beginning.

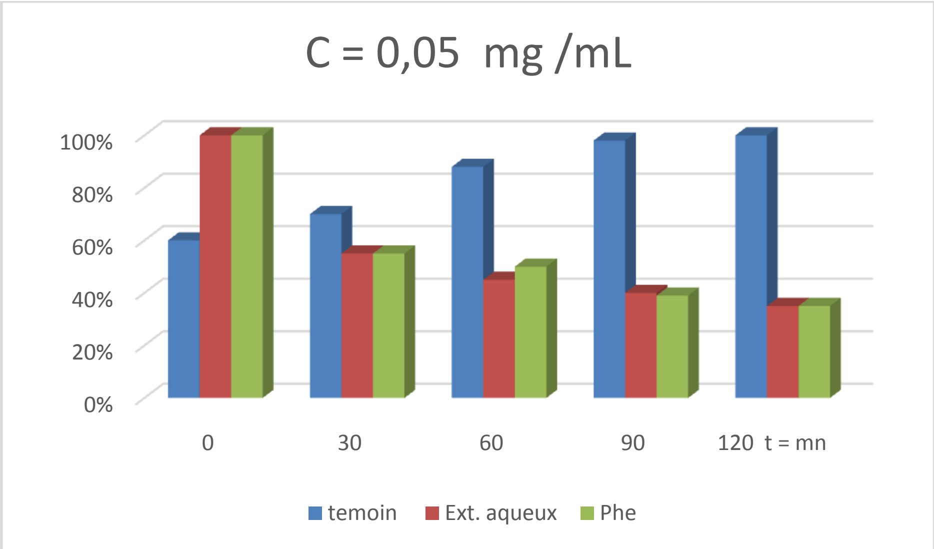

Figure 2: In vitro antisicklingactivity of the aqueous extract at the concentration: 0.05 mg / mL for the combination of the three plants.

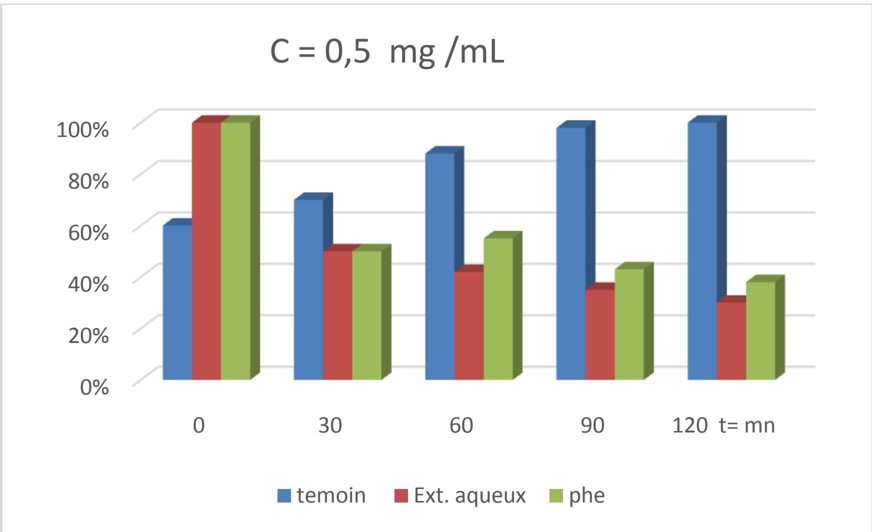

Figure 3: In vitro antisicklingactivity of the aqueous extract at the concentration: 0.5 mg / mL for the combination of the three plants.

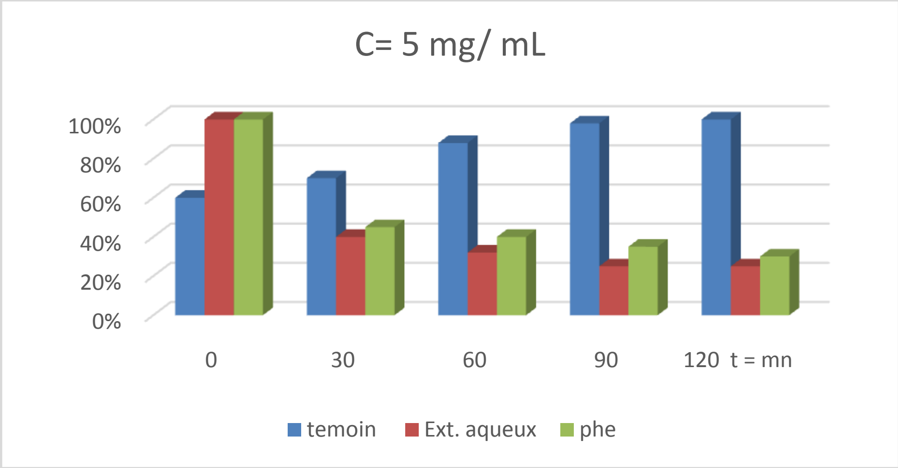

Figure 4: In vitro antisicklingactivity of the aqueous extract at the concentration: 5 mg / mL for the combination of the three plants.

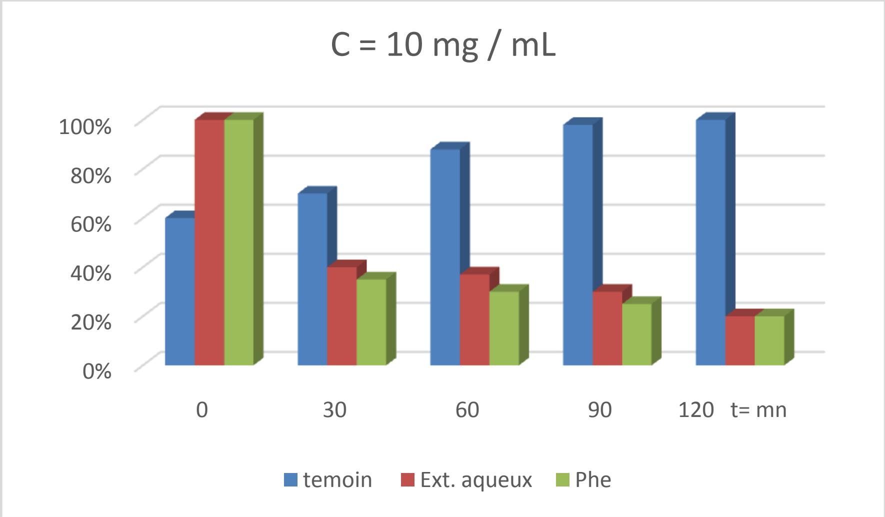

Figure 5: In vitro antisicklingactivity of the aqueous extract at the concentration10 mg / mL for the combination of the three plants.

IV. Discussion

Phytochemical Screening

Plants are a potential source of bioactive natural molecules. Analysis of the results of phytochemical sorting revealed the presence of Alkaloids, Polyphenols, Terpenoids and Heterosids. Note that Gallic tannins are not present in two plants.

Many studies have already shown the biological activities of some compounds found in the combination of plants. For example, alkaloids and polyphenols are antioxidant compoundswhichpromote the regeneration of tissues by trapping free radicals[13]. The tannins, decrease the permeability of the blood capillaries by reinforcing their resistance to hemolysis[14]. Flavonoids are able to modulate the activity of some enzymes[15], to modify the behavior of several cellular systems.They could have a several biological activity, including antioxidant, anti-inflammatory properties[16], antiulcer, antiallergic[17], anti-tumor, antimicrobial[18], antiviral[19.]

Taken individually the composition of *J. secunda*, *P. nigrescens* and *J. grossypiifolia* gives in part the results of the phytochemical sorting of the combination of the three plants. Our results are comparable to those of Gbenou, Mpiana[20, 21]who showed the presence of ALC +, POL + in leaves of J. secunda contained. The presence of ALC +, POL +, TS + has been confirmed in the aqueous extracts of *P. nigrescens* leaves[22, 23, 24, 25, 26, 27] .These same compounds were found in seeds, leaves and roots of *J. grossypiifolia* made byBiswanath[28]and Abiodun[29]. Apart from anthocyans, leucoanthocyanins, anthraquinon and cardiac glycosides which were not search in our study, the presence of large chemical groups was confirmed by these authors.

Antisickling activity

The analysis of histograms (2 to 5) shows that part, SS red blood cells. But in the presence of aqueous extracts of the plant combination the red blood cells of SS blood return to their normal form despite being in hypoxia conditions created by the addition sodium met bisulfite 2%. Water being one of the most solvents polar, this result shows that the active ingredients of this plant are polar chemical compound. This proves that the aqueous extract has antisickling activity in vitro.

It appears from this analysis that the aqueous extract has a better antisicklingactivity and is both growing and homogeneous. From this, we deduce that polar solvents such as water would best concentrate the compounds responsible for antisicklingactivity and these compounds are polar in nature[30]. A similar result of polar compounds with antisicklingactivity was obtained by Seck[31]by testing the methanolic extract of *Leptadeniahastata* roots on sickle-cell red blood cells.

Our results would be better than those obtained by Mpiana[32]and Sawadogo [33]. In fact, the results obtained in the two studies with the aqueous extract of *Jatrophacurcas* showed that this difference could be explained first by the species used by Mpiana and second by the choice of red blood cells (AS), the concentration (200 mg / mL) used by Sawadogo. This corresponds to 20 times the highest concentration of our study with a normal 80% of cells. Polymerization capacity is higher in SS red cells than AS red blood cells. However anthocyanin extract leaves of *Justiciasecunda* by Ngbolua[34]showed antisickling activity. As for Kade[35] and Imagna[19]; they showed that the activity of the leaves, roots and stems of *P. nigrescens* is due to the presence of flavonoids, alkaloids, tannins, glycosides, saponins and anthraquinon which have an inhibitory action on hemolysis of red blood cells. This would induce the stability of the erythrocyte membrane.

Of all the foregoing, it should be noted that the extracts of the combination of the three plants generate antisickling activity at all the concentrations tested. We can therefore say that the use of this combination of three plants to fight against sickle cell disease in traditional environment is justified.

Antioxidant properties of plants

Oxidative stress is one of the important points of sickle cell disease. He is involved in the phenomena of hemolysis, ischemia, vaso-occlusion, cell necrosis, bacterial infection and damage to various organs[36]. Oxidative stress is defined as an imbalance between pro-oxidants and antioxidants in favor of the first. Indeed, under physiological conditions, reactive oxygen species (ROS) produced continuously by the body are trapped by an effective antioxidant defense system that regulates their excessive production[37]. In sickle cell disease, the protein aggregates of band 3 are formed early, due to the auto-oxidation of hemoglobin S, the accumulation of iron in the erythrocyte membrane and lesions of the oxidizing membrane resulting in reduced life time of sickle cell[38].

Given the oxidative context well present in sickle cell disease, the issue of therapeutic development based on the administration of antioxidants is needed naturally.

However, in sickle cell patients, a weakening of their defense system due to a decrease in production and / or an excessive consumption of antioxidants comes to break this balance. Moreover, the use of synthetic molecules of antioxidants is called into question because of health risks. Continuous drug use may result in toxicity in sickle cell patients[39, 40]. Plants are an potential source of bioactive natural molecules, including alkaloids, terpenoids, steroids and polyphenols. Which are compound with antioxidant properties[41, 42]. Studies conducted by teams of researchers from Côte d'Ivoire and other countries have shown the antioxidant activity of some plants, including our plants.

The sickle-shaped red blood cells that can not cross the small vessels, they tend to hang by causing thrombosis in the organs. It follows a thrombosis which can lead to pulmonary superinfections with bacterial pneumonitis[41].

Susceptibility to infection or anti-infective property

Susceptibility to sickle cell infections is life-threatening for people with Sickle Cell Disease (SCD) in our resource-limited settings. Studies have shown that respiratory and bone infections, septicemia and malaria are most common among homozygous (SS) people. The pathogens encountered were streptococci, staphylococci, pneumococci, plasmodium, salmonellae and Haemophilus influenzae[43, 44].

These microorganisms develop some resistance to synthetic molecules in several regions of the world[45, 46]. Resistance to microorganisms is one of the most serious public health problems and is one of the leading causes of treatment failure in people with SCD.[47, 48]

So Medicinal plants are a significant source of new drugs; especially since they have fewer side effects[49]. Hence the interest of moving towards active molecules from plants. The synthesis of most scientific articles shows the antibacterial activity[50, 51], antimicrobial[26, 15, 22] antiviral[16] of several plants including our plants.

V. Conclusion

The establishment of a phyto drug is a current trend in the management of tropical and genetic diseases such as sickle cell anemia. This study was conducted with the aim of finding an alternative medicine to which the population will have immediate access and less expensive in Côte d'Ivoire.

The objective of this work was to verify in vitro the anti-sickle activity of the aqueous extract of the combination of J. secunda, P. nigrescens and J. grossypiifolia used in the treatment of sickle cell disease in Côte d'Ivoire.

It appears from this work that the extract has an activity on the reversion of red sickle blood cells. This activity is due to the presence of large chemical groups (alkaloids, polyphenols). Also, the antisickling, antioxidant and antimicrobial activities of these three plants could act either individually or in synergy, the need to carry out additional studies on the nature of the active compounds and on the determination of the new stress prophylaxis strategy to fight against the stress of oxidant and infections produced during sickle cell disease. These results could justify some traditional uses of these plants in the treatment of sickle cell disease.

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