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Bioactivity and Chemical Analysis of *Drepanoalpha*: An Anti-Sickle Cell Anemia Poly-Herbal Formula from Congo-Kinshasa

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Abstract

Sickle cell disease (SCD) is a genetic disease that is becoming a public health problem in sub-Saharan African countries. Phytotherapy seems to be an affordable approach for sickle cell patients in this region. In Democratic Republic of the Congo, a hundred of plants used in the management of SCD were studied through bio-guided assays. Three most active recipes were used for the formulation of an improved traditional drug, called Drepanoalpha**,** of which scientific evidence concerning the effectiveness and innocuousness is well established. The present study aimed to identify as well as to quantify the phyto-markers of biopharmaceutical relevance for the evaluation of the quality and the standardization of Drepanoalpha; to identify and quantify the nutrients contained in this phytomedicine and to determine its energy value; to assess its antisickling and antibacterial activities *in vitro*. The obtain results indicated that Drepanoalpha contains total polyphenols (289.3 ± 6.3 mg gallic acid equivalents /g), flavonoids (350.7 ± 1.3 mg equivalent of quercetin/g), anthocyanins (108.0 ± 5.6 mg D-catechin equivalent/g) and tannins (202.5 ± 0.3 mg tannic acid equivalent/g). It also contains glucids (55.3 g/100 g of drug), lipids (5.7 g/100 g of drug), proteins (16.6 g/100 g of drug) and micro-nutriments such as Zn, Mn, Fe (9.0 mg/100 g of dry matters), Mg (1.4 mg/100 g of DM), Ca (4. 8 mg/100 g of DM), K, and P as well as the vitamin C. Its energy value is about 1482 kJ/100 g of drug. The organic extracts displayed *in vitro* antisickling (rate of normalization >90%) and antibacterial activities (towards *S. aureus*) (CMI<500 µg/ml). Thus, it is desirable that flavonoids be targeted as sphyto-markers for the evaluation of the quality of Drepanoalpha and its standardization.

Keywords: Sickle cell disease; Drepanoalpha; Nutraceutical; Nutritional value; Democratic Republic of the Congo

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Introduction

Sickle cell disease (SCD) is a hemoglobinopathy due to the presence of hemoglobin S in the blood. Clinically, it is characterized by a vaso-occlusion, hemolytic anemia and increased susceptibility to infections [1]. The management of SCD is difficult in poor countries, especially in the Democratic Republic of the Congo (DRC) that possesses 2% of its population affected by this genetic disease. In DRC, where over 50% of territory consists of rainforest, research and development of improved traditional medicines (ITM) based on scientific evidence is a priority [2,3].

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According to the World Health Organization (WHO), more than 80% of African population resorts to plants and traditional medicine for primary health care [4,5]. This is the case of sickle cell anemia, a disease that affects mainly populations with low income. In DRC, a vast molecular bio-prospecting program has identified by bio-guided assays a hundred of antisickling plants [2,3,5-11]. Drepanoalpha is an ITM formulated with a number of food plants that have shown antisickling, anti-hemolytic, anti-radical and antioxidant activities. It possesses the properties to increase hemoglobin, platelets, red blood cells and white blood cells rates. Phytochemical studies have shown that anthocyanins, organic acids and its derivatives are the main active principles of Drepanoalpha [12,13]. Drepanoalpha has an LD_{50} greater than 4000 mg/Kg of body weight and does not interfere with the physiological functions of the liver and kidneys. The product is well tolerated in humans and caused significantly the disappearance of sickle cell crises for months and this is probably by a mechanism involving the epigenetic modulation of gene expression of fetal hemoglobin. The case of total disappearance of crises in subjects with SCD treated with Drepanoalpha is well established. It has been observed that for this purpose, treated patients showed the same phenotypic profile than normal individuals and predict the possibility of epigenetic modulation of gene β^S operating in these individuals [12]. This study was initiated with the overall goal to complete the phytochemical, pharmacological and biochemical data of Drepanoalpha. This work's specific objectives are to identify and assay phyto-markers of biopharmaceutical interest in order of the traceability and standardization of Drepanoalpha; to identify and assay the nutrients in this ITM and to determine its energy content; to assess its anti-sickling and antibacterial activity *in vitro*. The interest of this study is to enhance the Congolese ethno-medical knowledge based on the scientific evidence.

Materials and Methods

Biological materials

Phytomedicine Drepanoalpha is packaged in the form of powder in plastic bags of 100 g/bag.

Blood and bacterial strains: Blood samples used were taken from known sickle cell anemia subjects attending the "Centre de Médecine Mixte et d'Anémie SS" located in "Kalamu district", Kinshasa, 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. An informed consent was obtained from all the patients participating in the study and all the research procedures have received the approval of Department of Biology Ethics Committee. Two bacterial strains aged of 24 hours from the Laboratory of Bacteriology of Pharmaceutical sciences faculty, University of Kinshasa were tested. These strains are from *Escherichia coli* ATCC 27195 and *Staphylococcus aureus* ATCC 33591.

Methods

Phytochemical studies: Chemical Screening The chemical screening was performed according to the standard technique as previously described [5-9].

Fractional Extraction Fifty grams of Drepanoalpha powder were soaked for 48 hours in increasing solvent polarity (petroleum ether, ethyl acetate and methanol) respectively in the ratio of 1:10 (w /v). After filtration, the filtrates were concentrated on a rotary evaporator and evaporated to dryness in an oven at 40°C for 48 hours. Determination of phyto-markers content. Anthocyanins The determination of Drepanoalpha anthocyanin content is based on the protocol described by Adedapo et al. [14]. A volume of 0.5 mL of the extract (1 mg/mL) was taken and mixed with 3 mL of a 4% solution of vanillin-methanol (MeOH) and 1.5 mL concentrated hydrochloric acid and the mixture is incubated for 15 minutes. The absorbance is measured in a spectrophotometer (GENESYS 10S UV-Vis) at 517 nm. The anthocyanin content is expressed as D-catechin equivalent (mg/g) extending from a range of ten dilutions ranging from 100 µg/mL to 0.195 µg/mL. Methanol was used as a control. Flavonoids Drepanoalpha flavonoid content was determined spectrophotometrically according to Quettier et al. [15]. The reaction mixture contains 1 mL of ITM methanolic extract (2 mg/mL) and 1 mL of 2% $AlCl_3$ dissolved in MeOH and the whole was well stirred. After an hour of incubation at laboratory temperature (28 ± 1) and under shade, absorbance is read at 415 nm. The mixtures are prepared in triplicate for each analysis and the average value is maintained. This procedure is repeated for the standard solution of quercetin in eight different dilutions (ranging from 187.5 µg /mL to 1.464 µg/mL) for the establishment of the calibration curve. For the preparation of the blank, the methanol extract of Drepanoalpha was replaced with 1 mL MeOH. The content of flavonoid of the ITM is expressed in mg equivalent of quercetin per g of the dry extract of ITM. Total phenols The dosage of polyphenol in the ITM was performed using the Gutfinger method as previously described [16] with the Folin Ciocalteu-reagent. The reaction mixture was composed of 0.5 mL of methanolic extract of ITM (0.5 mg/mL); 2.5 mL of distilled water and 0.25 mL of Folin-Ciocalteu reagent. After 3 minutes, 1.0 mL of Na_2CO_3 20% saturated solution was added. Afterwards, the mixture was stirred and incubated at laboratory temperature and under shade for an hour. The absorbance was noticed at 725 nm. Each assay was repeated three times. The same procedure was followed for eight different dilutions of the standard solution of gallic acid from 5 to 150 µg / mL and the calibration line was thus constructed. For the control, the extract was replaced by MeOH. The amount of total polyphenols is expressed in mg of gallic acid equivalents (GAE) per g of dry extract. Tannins The content of Drepanoalpha in tannins was determined by the method described by Soetan [17]. Thus, 50 mg of dry extract was weighed and macerated in a solvent system consisting of 20 mL of acetone and 5 mL of glacial acetic acid for 5 hours for tannin extraction. The mixture was filtered using a coffee filter paper and the absorbance of the filtrate obtained is read at 720 nm. Tannic acid was used as standard and 9 dilutions in acetone-acetic acid mixture (ranging between 500 µg/mL and 9.765 µg/mL) were prepared. One mL of acetone was used as control. Analytical thin layer chromatography (TLC) Five µL of solution of 10 mg/mL of each extract is deposited using a capillary tube on a silica gel 60F254 plate of 10 x 10 / cm. The solvent was evaporated

after each deposition at ambient laboratory temperature. Thus, TLC plates were prepared and placed into chromatographic wells beforehand saturated with the mobile phase made of Ethyl acetate/formic acid/acetic acid/water (100:11:11:27; v/v) for migration. The TLC plates are analyzed in visible light and under UV (254 and 366 nm) before and after spraying with a solution of DPPH (2 mg/mL in MeOH).

Determination of Drepanoalpha macronutrient composition: Moisture The determination of moisture (expressed in g/ 100 g of ITM) was performed per the weight loss method [18] Fats Total lipids (expressed as g/100 g of ITM) were measured by Soxhlet method which consists in extracting under heat the lipids contained in the sample by means of an appropriated apolar organic solvent (n-hexane) [18]. Total ash the total ash (expressed in g/100 g ITM) were determined by incinerating a known amount of the sample in an electric muffle furnace until obtaining a white ash as previously described [18]. Crude fibers the raw materials or cellulosic fibers (expressed as g/100 g ITM) were measured following Kurschner method based on attack under reflux condenser, of Drepanoalpha powder by mixing acetic and nitric acids [18]. Total Proteins: Determining the crude proteins or total crude nitrogen content (expressed in g/100 g ITM) was performed according to Kjeldahl method [18]. Total Carbohydrates: The total carbohydrates were obtained by the method of Favier et al. [18]. It consists by subtracting from 100, the sum of the contents of the other constituents of the analyzed sample (moisture, protein, ash, and crude fiber). Energy value:The quantity of heat energy provided by 100 g of Drepanoalpha to a sickle cell patient was calculated using the method previously described [18] and amended by Congolese Control Office (OCC) according to the method found in the food codex book by applying Atwater coefficients for proteins, lipids and carbohydrates.

Determination of Drepanoalpha micro-nutrient composition

Qualitative Analysis: The minerals (Calcium, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium, Zinc) were determined from the acidified total ash (concentrated HCl) using specific chemical reagents: Fe(NH_4SCN 0.1M); Mg (Triethanolamine 20% and Black Eriochrome); Ca (NH_4OH and (NH_4) $2C_2O_4$); Zn (0.1N NaOH); K (Bunsen-beak flame); Na (Bunsen-beak flame); P (HNO_3 and molybdic liqueur); Mn (HCl and $AgNO_3$), Vitamin C ($KMnO_4$) [18]. Determination of some minerals (expressed as mg / 100 g of dry matters):The mineralized solution (MS) was prepared by dissolving 5 g of total ash in 500 mL of an acidified solution (HNO_3 10%) boiling for 3 to 5 minutes. After cooling, the solution was filtered using a Whatman paper N°1. The filtrate is the mineralized solution.

Biological studies: *In vitro* antisickling activity: The Emmel test was used as previously described [2,3,5-10], briefly the blood sample was mixed with plant extracts at different concentrations using physiological saline (0.9% NaCl) as dissolution solvent. The control consists of diluted sickle cell blood without extract. The effect of the various extracts is observed by optical microscopy after an exposure time between 24 and 48 hours under hypoxic and isotonic conditions in order to assess the persistence time of the antisickling effect. A digital camera was used to record microscopic images of obtained erythrocytes. Afterwards, these micrographs were then processed by MOTIC pictures 2000 version 1.3 software as previously described [5-9].

Antibacterial activity: The antibacterial activity was evaluated by micro-dilution method in liquid medium as previously reported [19-21]. The extract to be tested (20 mg) is first dissolved in 250 µL of DMSO and the final volume is adjusted to 5 mL along with the Mueller Hinton culture medium (final DMSO concentration is 5%). The bacterial suspension was prepared by mixing 2 mL of physiological saline, two single colonies of strains to be tested (*Escherichia coli* ATCC 27195 and *Staphylococcus aureus* ATCC33591) then incubated for 24 hours in order to obtain 0.5 McFarland (10^8 cells/mL). Afterwards, the bacterial suspension was then diluted in order to obtain 10^6 cells/mL (dilution 1/100). The micro-dilution test was performed in sterile microplates in polystyrene of 96 wells. Briefly, 100 µL of culture medium were placed inside wells (A_2 to A_8, B_2 to B_8, C_2 to C_8, D_2 to D_8, E_2 to E_8, F_2 to F_8 and then the 11th and 12th column were used as controls). With a micropipette, 200 µL of each extract to be tested (4000 µg/mL) were placed in A_1 and B_1 (extract 1: petroleum ether), C_1 and D_1 (extract 2: ethyl acetate) and E_1 and F_1 (extract 3: methanol) wells respectively. Then 100 µL of each of stock solution extract were taken in order to perform serial dilutions of 2 by 2 to the eighth column. The last 100 microliters (column 8) are eliminated, then 15 µL of inoculum (10^8 cfu/mL) were aseptically taken with a micropipette and transfer into all wells of the microtiter plate except wells of 11th column (culture medium sterility control). The wells of 12th column served as bacterial growth control (inoculum and growth medium). The plates were incubated in an incubator at 37°C for 24 hours. After this time, 5 µL of TCC (2, 3, 5 triphényltetrazoliumchloride) at 2% was added to each well and the microplates were then incubated for 15 minutes. The minimum inhibitory concentration (first wells showing no bacterial growth) was determined after 24, 48 and 72 hours.

Results

Chemical screening

Phytochemical markers: **Table 1** shows the contents of various Drepanoalpha extracts in phyto-markers. The values are averages of at least three treatments (mean ± standard deviation). It can be noticed from the **(Table 1)** that the total polyphenols, anthocyanins and flavonoids are more concentrated in the ethyl acetate extract while tannins are rather more concentrated in the methanolic extract. The analysis of variance (ANOVA) shows that the difference in the content of phyto-extracts markers is highly

Table 1 Mean values of phyto-markers content of Drepanoalpha.

Extracts	Total phenol (mg GAE/g)	Flavonoids (mg QE/g)	Anthocyanins (mg CE /g)	Tannins (mg TAE/g)
EEP	109.8 ± 25.0	81.8 ± 6.1	11.7 ± 2.0	55.8 ± 0.1
EAE	289.3 ± 6.3	350.7± 1.3	108.0 ± 5.6	101.3 ± 0.2
MeOH	226.0 ± 9.4	186.1± 14.2	64.9 ± 4.6	202.5 ± 0.3

EEP: petroleum ether extract; EAE: ethyl acetate extract; MeOH: methanolic extract; QE: Quercetin equivalent; GAE: Gallic acid equivalent; CE: D-catechin equivalent; TAE: tanic acid equivalent.

significant ($p<0.05$). The phytochemical profile of three extracts is shown by the analytical chromatogram (TLC) **(Figure 1)**. The chromatographic profile revealed the presence of yellow spots well resolved in methanol extract of Drepanoalpha indicating the reduction of DPPH radical **(Figure 1**).

Biochemical composition: Macronutrients and energy value: The biochemical composition of Drepanoalpha (g/100 ITM) and its energy value (kJ/100 g of ITM) are presented in **Table 2**. It is shown from this table that Drepanoalpha contains Mg, Ca, Zn, K, P, Mn and vitamin C. The quantitative analysis of three minerals gives (in mg/100 g of dry matters) 4.80 (Calcium); 1.41 (Magnesium) and 9.04 (Iron).

Biological activities: *In vitro* antisickling activity: Figure 2a-2d gives the optical micrographs of sickle cells untreated (control) and treated with Drepanoalpha extracts.

As it can be seen in **Figure 2a**, almost all the erythrocytes in the microscopic field are of sickle cell phenotype. This sufficiently proves that the blood used is actually from a sickle subject. On the contrary, in the presence of petroleum ether, ethyl acetate and methanolic extracts, the red blood cells returned into their normal biconcave shape (**Figure 2b-2d).**

Antibacterial activity: The results of antibacterial activity of Drepanoalpha extracts are shown in **Table 3** From this table, it appears that only *S. aureus* is sensitive to Drepanoalpha extracts (MIC<500 µg/mL) [21].

Discussion

Chemical screening

The phytochemical screening revealed the presence of various secondary metabolites such as polyphenols, flavonoids, tannins, anthocyanins, saponins, quinones, leucoanthocyanins, alkaloids and terpenoids. These results confirm that of previous work [13]. Anthocyanins and organic acids had been indicated as responsible of antisickling activity of some plants used in Congolese traditional medicine in the management of sickle cell disease [2,3,5-10]. The presence of terpenoids in Drepanoalpha could facilitate the absorption of anthocyanins into sickle cell [22]. It should be noticed that phenolic compounds including flavonoids, which are abundant in Drepanoalpha can be chosen as phyto-markers for quality control and standardization of this nutraceutical.

Biochemical composition

Macronutrients: The content of Drepanoalpha in total proteins, crude fibers and total ash is superior to that of Vamine, a food supplement made from traditional food resources of Bandundu in the DRC [23]. It is well established that a diet rich in protein significantly reduces the circulating levels of inflammatory

Table 2 Biochemical composition of Drepanoalpha (g/100 g ITM).

Macronutriments	Quantity (%)
Moisture (Humidity)	11.71 ± 1.04
Crude Proteins	16.64 ± 1.00
Total ash	12.62 ± 0.38
Fats	5.70 ± 0.70
Crude Fibers	5.96 ± 0.27
Hydrates of carbone	55.33 ± 1.73
Energy value	1482 kJ/100g

Table 3 Qualitative analysis of Drepanoalpha + indicates the presence of the desired element.

Micronutriments	Results
Iron (Fe)	+
Magnesium (Mg)	+
Calcium (Ca)	+
Zinc (Zn)	+
Potassium (K)	+
Phosphorus (P)	+
Manganèse (Mn)	+
Vitamine C (Vit. C)	+

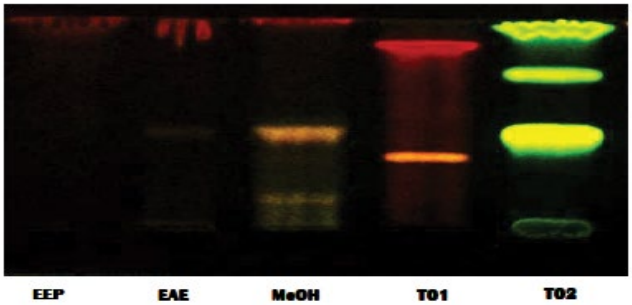

Figure 1 $T0_1$: Rutin hypericinée; $T0_2$: Chrorogenic Acid; EEP: petroleum ether extract; EAE: ethyl acetate extract; MeOH: Methanol extract.

Chromatographic profile of extracts from Drepanoalpha at 366 nm. Eluent (ACOEE/CH_3COOH/HCOOH/H_2O 100: 11: 11: 27; v/v /v).

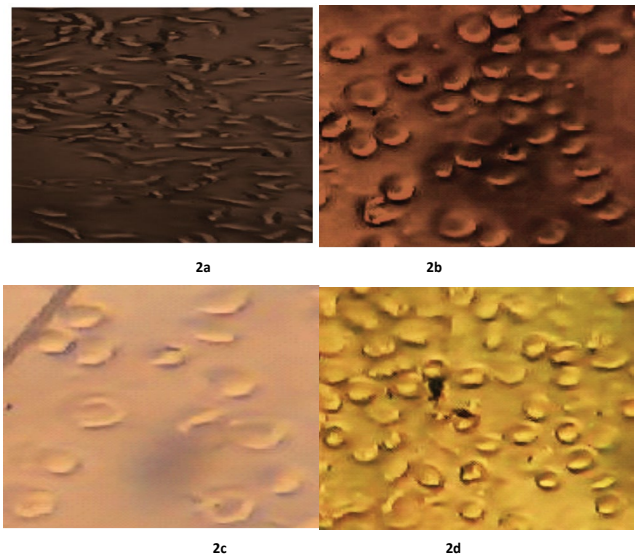

Figure 2 **a** Phenotype of untreated sickle cells (control), **b** Phenotypes of treated sickle cells with 50 µg/mL of petroleum extract, **c** Phenotypes of treated sickle cells with 50 µg/mL of ethyl acetate extract, **d** Phenotypes of treated sickle cell with 50 µg/mL of methanolic extract (NaCl 0.9%, $Na_2S_2O_5$ 2%, x500).

proteins and interleukin 6 (IL-6) in sickle cell patients [24]. Futhermore, this ITM showed higher fats content than cereals, cowpea, Bean, vegetables and fruits used in the traditional diet at the same Province [18]. These results indicate that Drepanoalpha can be used as food supplement because of its energy and its content of macronutrients. This nutraceutical can allow patients to compensate their daily needs during treatment by providing the plastic and energetic needs and protection needs covered by the mineral salts and trace elements. The presence of crude fibers in this ITM shows how it will favor digestion by increasing the production of stools; colic fermentation stimulation; the reduction of serum cholesterol and glycemia and/or postprandial insulin [23].

Micronutrients: The high content of iron in Drepanoalpha as a hematopoietic factor would prevent anemia in sickle cell patients. The presence of zinc in this nutraceutical, which is a cofactor of antioxidative enzymes would help to strengthen the immune system of patients, and would prevent against bacterial infections. In fact, several consequences in terms of health have been reported in case of zinc deficiency, including immune system dysfunction, abnormal or delayed sexual maturation, low power healing, decreased activity, and the level of Expression of zinc metalloproteins. However, it has been shown that zinc supplementation increased in sickle cell subjects, the activity of natural killer cells (NK cells) and the ratio of CD^{4+} / CD^{8+} [25-29]. Thus, Drepanoalpha as a food supplement would boost the immune system of sickle cell patients. It is also well established that the copper content is very high in the serum of sickle cell disease patients, zinc supplementation would lead to a hypo-copper state [30]. Zinc also acts as an antagonist in the cytoplasmic calcium retention process, in stabilizing the erythrocyte while preventing hemolysis (inhibition of the opening of Gardos channel). It also has anti-oxidative properties and is the cofactor for several oxidative enzymes such as catalase, glutathione-S-transferase and alkaline phosphatase and more than 200 zinc dependent enzymes [31]. This metal is also an inhibitor of calmodulin and is endowed with antisickling activity along with inhibitors of calmodulin such as Cetidel for which activity is well established [32]. Thus, the antisickling and anti-oxidative properties of Drepanoalpha would also be partly due to the presence of zinc. Sickle cell disease is associated with micronutrient deficiency (minerals, trace elements and vitamins C, E, D, etc.) [25] and particularly the erythrocyte concentration of magnesium of sickle cell patients are known to be low [25,28]. Indeed, the low intracellular concentration in magnesium is associated with intracellular sickling of erythrocytes [28]. So, Drepanoalpha will allow compensating the mineral ion content in patients and would prevent the cellular dehydration and potassium leak as the Gardos channel inhibitor [5]. Due to its content in vitamin C, this ITM would also prevent the formation of Heinz bodies (denatured hemoglobin) and promote the absorption of iron [29]. In African population, mainly Congolese population where some nutritional problems is found, the treatment of sickle cell patients with Drepanoalpha would help by providing some essential nutrients.

Biological activities: *In vitro* antisickling activity: Obtained results show that different fractions of Drepanoalpha have antisickling properties. Indeed, Drepanoalpha prevents erythrocyte from sickling in hypoxic conditions indicating it antisickling activity and confirming the results of previous works on this ITM [13]. Drepanoalpha has shown antisickling, anti-hemolytic, anti-radical and antioxidant activities. It has properties to increase red blood cells level, hemoglobin rate, platelets and white blood cells [13]. In addition, a multicenter pilot study confirmed that Drepanoalpha increases hemoglobin rate and sickle cell crises in the treated patients disappear [12,13]. In Africa, due to the lack of access to modern health care, inadequate and poor distribution of modern health workers as well as the socio-cultural behavior, more than 80% of the population turns to medicinal plants for healing. So, research and development of IMT based on scientific evidence is crucial [4]. Drepanoalpha would facilitate patients to access to care at lower cost and would improve the prognosis of sickle cell disease as well as the life expectancy in low income population areas.

Antibacterial activity: Only the Gram positive bacterium *S. aureus* is sensitive to Drepanoalpha extracts when the Gram negative *E. coli* is not sensitive **(Table 4).** The sensitivity of Gram-positive bacteria could be attributed to their outer peptidoglycan layer which is not an effective permeability barrier. Gram-negative bacteria possessing an outer phospholipidic membrane carrying the structural lipopolysaccharide components make the cell wall impermeable to lipophilic solutes while porins constitute a selective barrier to hydrophilic solutes with an exclusion limit of 600 Da [19,20]. This antibacterial activity of Drepanoalpha would be due to the phenolic compounds present in its extracts. These results corroborate previous studies on the antimicrobial properties of secondary metabolites of plant origin [19-21].

Conclusion

The purpose of this study was to identify few phyto-markers contained in Drepanoalpha, determining its biochemical composition of macro and micronutrients in one hand, and on the other hand, to assess its antibacterial and antisickling properties. The results show that polyphenols, flavonoids and anthocyanins are more abundant in the ethyl acetate extract when tannins are more concentrated in the methanolic extract. Drepanoalpha contains carbohydrates, lipids, proteins and micronutrients such as Zn, Fe, Mn, Mg, Ca, K, and P as well as vitamin C. Its

Table 4 Effects of Drepanoalpha extracts on bacterial growth *in vitro* (micro-dilution method; dye: TCC 2%).

Concentration (µg/mL)	*E. coli* ATCC 27195			*S. aureus* ATCC 33591		
	EEP	EAE	MeOH	EEP	EAE	MeOH
4000	-	-	-	-	-	-
2000	-	-	-	-	-	-
1000	+	-	-	-	-	-
500	+	+	+	-	-	-
250	+	+	+	-	-	-
125	+	+	+	+	+	+
CMI (µg/mL)	>1000	1000	1000	250	250	250

+: Bacterial growth: TCC conversion (Colorless) red formazan; -: No visible growth (the well color is the one of the extract); MIC: minimum inhibitory concentration; E. coli: Escherichia coli; S. aureus: Staphylococcus aureus; EEP: petroleum ether extract; EAE: ethyl acetate extract; MeOH: Methanol extract

energy value is of 1482 kJ/100 g. All the Drepanoalpha extracts showed *in vitro* antisickling and antibacterial activity (against *S. aureus*). Therefore, a chromatographic study for quality control of different batches of Drepanoalpha is needed by targeting flavonoids as phyto-markers. The content of Drepanoalpha antinutritional factors such as oxalates, phytates, cyanides, nitrites be determined as well as its amino acid and fatty acid composition require further studies.

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