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Antioxidant Activity, Acute Toxicity and Anti-Sickle Cell Properties of *Spathodea campanulata* P. Beauv. (Bignoniaceae)

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Abstract | **Original Research Article**

Oxidative stress is one of the main causes of several diseases such as sickle cell disease. The aim of this study was to investigate the pharmacological effect of four anti-sickle cell plant recipes. The extracts were obtained after decoction and maceration by homogenisation. Phytochemical was carried out using colorimetric methods. Phenolic and alkaloid content was determined using specific reagents. Antioxidant activity was assessed using DPPH and FRAP methods. Acute toxicity was studied according to OECD 423 and the antisickling effect of the extracts was studied using Emmel's method. The results obtained showed that the four (4) plant recipes contain amino acids, alkaloids, phenolic compounds, saponins and sterols. The total phenolic compound contents were observed with the DbA extract recipes (36.71 ± 0.34 mg EAG/g DM; 20.20 ± 0.92 mg EQ/g DM; 31.12 ± 1.55 mg ECa/g DM) and ZHm (43.31 ± 0.92 mg EAG/g DM; 19.76 ± 1.99 mg EQ/g DM and 26.57 ± 1.6 mg ECa/g DM). Total alkaloids were more present in the JJP recipe (36.11 ± 0.16 % E.Atropine/g DM). DbA extracts (IC50 = 10.67 µg/mL and 6.45 µg/mL) generated better anti-free radical activities. On the other hand, the JJP recipe extracts caused immediate mortality at single doses of 300 and 2000 mg/kg bw. After 120 min, the percentages of inhibition were 77.09% for ESc, 72% for DSc and 83.65% for phenylalanine in the presence of 10 mg/mL of the substances tested. These results illustrate the wealth of bioactive compounds found in medicinal plants, making it essential to study the toxicity of these products before consuming them.

Keywords: Antioxidant Activity, Acute Toxicity, Anti-Sickle Cell Properties, *Spathodea Campanulata*.

1. INTRODUCTION

Sickle cell disease is the most common and severe haemoglobinopathy caused by a single genetic mutation resulting in repeated polymerisation and depolymerisation of haemoglobin. This alternation in haemoglobin shape causes intravascular haemolysis, cell adhesion vascular occlusion [1]. Haemolysis causes indirect oxidative damage by generating reactive oxygen species through various pathophysiological mechanisms. These reactive oxygen species, which are present in large quantities in the body, cause an imbalance between pro-oxidants and antioxidants in favour of the former; this is oxidative stress [1]. Red blood cells have an integrated antioxidant system comprising enzymes as well as vitamins C, E and glutathione, which help them to fight oxidative damage. However, these antioxidants may not be sufficient to prevent the effects of oxidative stress in sickle cell patients [2]. In sickle cell disease patients, a weakening of their defence system due to reduced production and/or excessive consumption of antioxidants upsets this balance. In addition to haemolysis, haemoglobin is capable of self-oxidation, leading to the formation of methaemoglobin $HbFe^{3+}$ by oxidation of Fe^{2+} to Fe^{3+} in the presence of excess oxygen [3]. These self-oxidation of haemoglobin generates free radicals, including superoxide anions O_2^-. Although this phenomenon occurs in both Hb A and Hb S, studies have shown that the latter has the capacity to self-oxidation 1.7 times faster than Hb A [4, 3]. In addition, methaemoglobin, in the absence of iron reduction by methaemoglobin reductase, will denature into haemachromes. These haemachromes, present at very high levels in the erythrocyte membrane of sickle cell red blood cells, will promote oxidation of the membrane constituents of red blood cells [3]. It is therefore necessary to develop therapies targeting oxidative stress and restoring the redox balance in patients with sickle cell disease [1].

A number of research studies have therefore been carried out in this area, mainly in animal models [5]. However, few clinical trials are available, mainly at stage 3, which limits the real evaluation of these antioxidant therapies in sickle cell disease. *In vitro* studies have shown that non-essential amino acids stimulate glutathione production, significantly reducing the formation of sickle cell red blood cells and restoring their biconcave shape [6]. However, despite these promising results, studies testing the benefits of antioxidants have also produced mixed results, with Arruda and Co-workers demonstrating that the use of vitamins C and E increases markers of haemolysis [7].

Sickle cell disease causes significant morbidity and impairs quality of life, as well as straining healthcare systems wherever it occurs [8, 9]. In sub-Saharan Africa, it can contribute up to 90% of under-5 mortality [10], with around 500 children with sickle cell disease continuing to die prematurely every day [11]. This is due to late diagnosis and/or lack of access to comprehensive care, a trend that urgently needs to be reversed [12].

The incidence of sickle cell disease is very high in Africa, so it is necessary to adopt both natural and modern medicine. The exploration of indigenous African plants for medicinal purposes has long been the subject of extensive research [13]. This is due to infrastructural, financial and human constraints, which are generally unavailable in Black Africa, where disease is more common. In addition, the difficulty of accessing pharmacies, the cost of drugs and the need for lifelong treatment makes difficult for low-income rural populations to take painkillers. Côte d'Ivoire is no exception to this situation. As a result, there is renewed interest in medicinal plants for the treatment of sickle cell disease [13]. Many plant recipes are used in the treatment of sickle cell disease in Africa as functional alicaments or improved traditional medicines. These include DREPANOSTAT® [14], NICOSAN® [15], FACA® [16], DREPANOALPHA® [17]. Traditional medicine based on scientific evidence can thus be an asset for universal health coverage (UHC) in Côte d'Ivoire with a view to achieving the United Nations' Sustainable Development Goal 3: "Health and well-being for all" by 2030. In order to contribute to the development of local resources for the management of chronic diseases such as sickle cell anaemia, this work will involve studying the pharmacological effect of four anti-sickle cell plant recipes.

2. MATERIALS AND METHODS

2.1. Materials

Animal Material

The animal material consisted of female rats of the species Rattus norvegicus (Muridae) of Wistar strain. These animals came from the vivarium of the Ecole Normale Supérieure (ENS) in Abidjan. They were used to study the acute oral toxicity of the aqueous extract and the hydroethanolic extract of the plant recipes.

Human Material

The blood sample used to assess the antisickling activity of *Spathodea campanulata* p. Beauv was taken from sickle cell patients with HbSS genotype who gave written informed consent on ethical grounds. Age and gender were not taken into account when sampling volunteers.

Inclusion Criteria

This study included sickle cell patients who met the following criteria:

- Patients with the SS genotype.
- Patients who had not received a blood transfusion two months prior to the blood test.
- Patients with a foetal haemoglobin level of less than 5%.

Exclusion Criteria

Patients meeting the following criteria were not included.

- Patients transfused two months prior to blood sampling.
- Homozygous patients with a foetal haemoglobin level greater than 5%.
- Patients having a seizure and/or other medical conditions.

Plant Material

The fruits of *X. aethiopica* (Dunal) A. Rich and *A. melegueta*, then the barks of *Z. leprieurii* (GUILL), *S. campanulata* p. Beauv, *D. benthamianus* and the leaves of *H. madagascariensis* (LAM), *J. gossypiifolia, J. secunda* and *P nigrescens* were collected in January 2018 in the Indénié-Djuablin region in eastern Côte d'Ivoire. For each batch of a given species, foreign species were eliminated. With the exception of the peels, the fruits and leaves were rinsed, shaken and cut into small pieces, then dried in the shade at room temperature (25-30°C) for four weeks. With the exception of the fourth recipe, which is monospecific, the other recipes are poly-specific. Indeed, the powders obtained after pulverisation of these plants were assembled at a rate of 25 g per plant for the recipe (JJP); 33.33 ± 1 g per plant for the second recipe (ZHm); 50 g per plant for the third recipe and 100 g of the fourth recipe (Sc). The different recipes were composed of four (4), three (3), two (2) and one (1) plant per recipe respectively.

2.2. METHODS

2.2.1. Preparation of Extracts

2-1-1-1. **Preparation of Decocts**

Following the modified method of Konkon [18], one hundred grams (100 g) of the plant powder mixture was boiled for 10 min in 2 L of distilled water. The decoctate was cooled to room temperature (25°C) and filtered three times through cotton wool and once through 3 mm Whatman filter paper. The filtrate was then dried at 50°C using Venticell® oven. The decocts obtained were coded:

- (DJJP) composed of *A. melegueta, J. gossypiifolia, J. secunda* and *P. nigrescens*.

- (DZHm) composed of *X. aethiopica, Z. leprieurii and H. madagascariensis*.
- (DDbA) composed of *A. melegueta and D. benthamianus*.
- (DSc) composed of *S. campanulata*.

2-1-1-2. **Preparation of the Hydroethanolic Extract**

The hydroethanolic extract was prepared according to the method of Zirihi [19]. One hundred grams (100 g) of the plant powder mixture was dissolved in one litre of hydroalcoholic solvent comprising 70% ethanol and 30% distilled water. The mixture was then homogenised using a Severin® brand blender. The homogenate obtained was wrung out in a cloth square and then filtered three times on cotton wool and once on whatman paper (3 mm). The filtrate was evaporated at 45°C in Venticell® oven for 24 hours. The 70% hydroethanolic extracts obtained were coded:

- (EJJP) composed of *A. melegueta, J. gossypiifolia, J. secunda and P. nigrescens.*
- (EZHm) composed of *X. aethiopica, Z. leprieurii and H. madagascariensis*.
- (EDbA) composed of *A. melegueta and D. benthamianus*.
- (ESc) composed of *S. campanulata.*

2.3. Phytochemical

2.3.1. Qualitative Research into Aromatic Amino Acids

Aromatic amino acids were determined by the xanthoprotein reaction. To 3 mL of extract, 1 mL of concentrated nitric acid was added. The tube was then boiled for 2 minutes. The appearance of a yellow colour indicates a positive reaction, revealing the presence of aromatic nuclei (benzene ring); amino acids from the aromatic series: tyrosine, tryptophan and phenylalanine. The test was performed according to the protocol developed by Fofana [20].

2.3.2. Qualitative Research into Secondary Metabolites

The qualitative search for secondary metabolites was carried out according to the method used by Nemlin and Brunel [21]. The phytochemical compounds sought and the reagents used are shown in Table I.

Table I: Reagents and tests for characterising chemical groups

Chemical groups	Reagents	Characteristic reactions
Alkaloids	Dragendorff Bouchardât	Precipitate or orange coloration Reddish-brown precipitate
Polyphenols	Ferric chloride	Blackish blue colour
Flavonoids	Cyanidine	Pink-orange precipitation
Sterols and polyterpenes	Liebermann	Green ring
Tanins	Stiasny	Precipitated in large flakes
Quinonic substances	Bornstraëgen	Red or violet colouring
Leucoanthocyane	Hydrochloric acid	Cherry-red or purplish discolouration
free anthraquinones	ammonium hydroxide	More or less red colouring
Saponins	Agitation	10 cm high persistent moss

2.3.3. Determination of Phenolic Compound Contents

2-1-1-3. ***Determination of Total Phenols***

Total phenols were determined using the Folin-Ciocalteu method Singleton [22]. One (1) ml of extract from the mixture of the four plants was introduced into a test tube. To the contents of the tube, 1 ml of Folin-Ciocalteu reagent was added. The tube was left to stand for 3 minutes, then 1 ml of 20% (w/v) sodium carbonate solution was added. The contents of the tube were made up to 10 ml with distilled water. The tube was placed in the dark for 30 min and the absorbance was measured at 745 nm against a blank. A standard range established from a solution of gallic acid (1 mg/mL), under the same conditions as the assay, was used to determine the quantity of phenols in the sample.

2-1-1-4. ***Determination of Total Flavonoids***

Flavonoids were assayed as described by Meda [23]. The extract of the mixture of the four plants (0.5 mL) was introduced into a test tube. Next, 0.5 mL distilled water, 0.5 mL aluminium chloride, 0.5 mL potassium acetate and 2 mL distilled water were added successively. The tube was left to stand for 30 min in the dark and the absorbance was read at 415 nm against a blank. A standard range established from a solution of quercetin (0.1 mg/mL), under the same conditions as the assay, was used to determine the quantity of flavonoids in the sample.

2-3-3-3. Determination of Tanins

Tanin content was determined using the method described by Bainbridge [24]. One (1) ml of extract from the mixture of the four plants was introduced into a test tube. Next, 5 ml of vanillin was added. The tube was left to stand for 30 minutes in the dark and the absorbance was read at 500 nm against a blank. The quantity of tanins in the samples was determined using a standard range established from a solution of tannic acid (2 mg/mL) under the same conditions as the test.

***2-3-3-4. Determination of* Total Alkaloids**

The assay was performed using the spectrophotometric method described by Sreevidya and Mehrotra [25]. A quantity of 5 mL of extract solution was taken and the pH was maintained between 2 and 2.5 with dilute HCl. 2 mL of Dragendorff's reagent was added and

the precipitate formed was centrifuged. The centrifugate was checked for complete precipitation by adding Dragendorff's reagent and the centrifuged mixture was decanted completely. The precipitate was washed with alcohol. The filtrate was discarded and the residue was then treated with 2mL of di-sodium sulphate solution. The brownish-black precipitate formed was then centrifuged. Completion of precipitation was checked by adding 2 drops of disodium sulphate. The residue was dissolved in 2mL of concentrated nitric acid, warming if necessary. This solution was diluted to 10mL with distilled water. Then 1mL of this diluted solution was taken and 5mL of thiourea solution was added. The absorbance was measured at 435 nm. The standard curve was made from a 10mg/L stock solution of atropine with a range from 0 to 1mg/mL. Absorbances were read using a spectrophotometer at 435 nm against the white tube prepared under the same conditions by replacing the extract with distilled water. The alkaloid content of the extracts (organs and liquid culture medium) was evaluated using the linear regression line and expressed in gram equivalents of atropine per 100g of powder or 100mL of liquid medium.

2.3.4. Assessment of Antioxidant Activity *in Vitro*

2-3-4-1 DPPH free Radical Scavenging Test

DPPH (2, 2 diphenyl-1-picrylhydrazyl) is generally the most widely used substrate for rapid and direct assessment of antioxidant activity due to its stability as a free radical and the simplicity of analysis. It absorbs in the visible at a wavelength of 517 nm. The experimental protocol used to study the free radical scavenging activity of DPPH was that described by **Parejo** [26], with some slight modifications.

2-3-4-2 Determination of Reducing Power

The reducing power of plant extracts was determined as described by Yildirim [27]. It was measured by the increase in absorbance at 700 nm of Perl Prussian blue due to the transformation of Fe^{3+} into Fe^{2+}.

2.3.5. Acute Toxicity

The toxicity test was carried out using the "dose adjustment" method of OECD guideline 423 [28]. The different doses of 300 and 2000 mg/kg bw of the compounds recipes were administered once to female rats and clinical signs of toxicity were noted.

2.3.6. Sickling Inhibitory Activity

The method is that of Emmel [29], using the protocol described by Imaga [30]. This method demonstrates the sickling inhibitory activity. It was used to assess the antisickle cell activity of plant extracts. The HbS genotype blood sample was washed by centrifugation for 5 min at 1500 rpm. The supernatant was removed using a Pasteur pipette, and the red blood cells were suspended in 0.9% NaCl saline. Next, 50 µL of this red blood cell solution was mixed with 50 µL of 2% sodium meta-bisulfite solution and 50 µL of plant extract, at concentrations of 2.5; 5 and 10 mg/mL. Each experiment was performed in triplicate. Phenylalanine (2.5, 5 and 10 mg/mL) was used as a reference. Morphological analysis of the erythrocytes was carried out using an immersion microscope, 30 min after addition of the plant extracts to the red blood cell-metabisulphite mixture up to 120 min. A drop of each mixture, between slide and coverslip, was observed using the SQA-Vision microscope. This made it possible to determine the rate of sickle cells and normal cells in each field. This activity was determined by the following formula: AA = (P0-Px) / P0*100 AA refers to the sickle cell inhibitory activity; P_0 the mean sickle cell count in the control; P_x the mean sickle cell count on the test slide in the presence of plant extracts at T_x = 0, 30, 60, 90 and 120 minutes.

Data Analysis

Statistical analysis of the data and graphical representation were carried out using Graph Pad Prism 9.0 software (San Diego, California, USA). Values were given as means followed by the standard error of the mean (M ± SEM). The difference between the two values was given by Dunnet and Turkey tests as post-tests. The significance level was set at $P < 0.05$ for the expression of the results.

3. RESULTS

3.1. Aromatic Amino Acid Composition

The intensity of the yellow colour after the tests carried out on the JJP, ZHm, DbA and Sc extracts, made it possible to detect the presence of aromatic amino acids in the ZHm, DbA and JJP extracts and their absence in the Sc extracts (Table II).

3.2. Phytochemical Composition of Recipes

The qualitative phytochemical analysis carried out on the extracts from the plant recipes revealed the presence of various groups of secondary metabolites. The results reveal the presence of polyphenols, tanins, flavonoids, alkaloids, leuco-anthocyans, steroids and triterpenes. Quinone substances were present in ZHm extracts, but were absent in extracts from JJP and DbA recipes (Table III). Anthraquinones were only present in the JJP hydroethanolic extract and the ZHm extracts. As for saponins, they were only present in the decocts of the extracts studied.

3.3. Phenolic Compound Contents of Recipes

3.3.1. Total Phenol Contents

The total phenol content of each extract is expressed in milligram equivalents of gallic acid per gram of dry matter (mg EAG/gMS), for which the equation of the calibration curve is Y= 0.0863x; R2 = 0.9977. Analysis of the results shows that the total polyphenol contents are 14.8 ± 0.99; 36.71 ± 0.34; 43.31 ± 0.92 and 4.44 ± 0.07 mg EAG/g dry matter for DJJP, DDbA, DZHm and DSc respectively. The decoctate from the ZHm recipe (DZHm) is richer in phenolic compounds (43.31 ± 0.92 mg EAG/g). There was a

significant difference (P < 0.0001) between the DZHm decoctate and the decocts from the DJJP and DSc recipes. The difference between the ZHm decoctate and that of the DbA recipe is P < 0.01.

The results for the hydroethanolic extracts were 16.84 ± 1.5, 33.82 ± 0.6, 24.16 ± 0.64 and 10.17 ± 0.49 mg EAG/g dry matter respectively for EJJP, EZHm, EDbA and ESc. EZHm was significantly richer (P < 0.0001 and P < 0.001) in total phenols than EJJP and EDbA. From a statistical point of view, a significant difference (P < 0.05) was observed between the aqueous and hydroethanolic extracts of the ZHm recipe, and a significant difference (P < 0.001) between the aqueous and hydroethanolic extracts of the DbA recipe. However, no significant difference was observed between extracts from the JJP recipe (Figure 1A).

3.2.2. Total Flavonoid Contents

The results obtained after flavonoid determination in the various recipe extracts are shown in Figure 1B. The calibration curve with equation Y = 1.3574x - 0.0181; R2 = 0.9976 obtained with quercetin was used to calculate the total flavonoid concentrations in the various plant extracts. The total flavonoid content is variable and differs from one recipe to another and from one extract to another. The total flavonoid content of the aqueous extracts was 8.83 ± 0.75 mgEQ/g for DJJP; 17.07 ± 1.54 mgEQ/g for DZHm; 13.73 ± 0.21 mgEQ/g for DDbA and 17.01 ± 3.6 mgEQ/g for DSc mgEQ/g. A significant difference (P < 0.0001) was observed between DJJP, DZHm and DSc. No significant difference was observed between the flavonoid content of DJJP and DDbA.

For the hydroethanolic extracts, the contents were 7.70 ± 0.2, 19.76 ± 1.15, 20.2 ± 0.64 and 22.45 ± 1.04 mgEQ/g respectively for the JJP, ZHm, DbA and Sc recipes. The total flavonoid contents of ESc and EDbA were significantly higher (P < 0.001) than those of EJJP and EZHm.

3.3.3. Total Tanin Contents

Total tanin contents were 4.88 ± 0.04, 8.64 ± 0.02, 12.12 ± 0.1 and 4.67 ± 0.85 respectively for the decocts of DJJP, DDbA, DZHm and DSc. For the hydroethanolic extracts, the contents were (EJJP: 8.33 ± 0.14 mg ECat/g); (EZHm: 26.57 ± 1.6 mg ECat/g); (EDbA: 31.12 ± 1.55 mg ECat/g) and (ESc: 6.12 ± 0.85 mg ECat/g). A significant difference (p<0.0001) was observed between DZHm and EZHm. In general, both extracts extracted phenolic compounds at varying levels (Figure 1C). Analysis of these results reveals that all the extracts (decocted and hydroethanolic) are rich in phenolic compounds at varying levels. The standard curve of equation y=3.92x; R2 = 0, 9727 allowed to determine the content of tanins.

Table II: Presence of aromatic amino acids

RECIPES EXTRACTS								
	JJP		**ZHM**		**DbA**		**SC**	
	DJJP	EJJP	DZHm	EZHm	DDbA	EDbA	DSC	ESC
Aromatic amino acids	+	+	++	++	++	++	-	-

Table III: Results of the phytochemical carried out on the various plant extracts

Recipes Metabolites	***JJP***		***ZHm***		**DbA**		***SC***	
	D	**E**	**D**	**E**	**D**	**E**	**D**	**E**
Sterol / Terpene	+	+	+	+	+	+	+	+
Polyphenol	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Leuco-anthocyane	+	+	+	+	+	+	-	-
Tanins Catechism	+	+	+	+	+	+	+	+
Gallic	-	-	+	+	-	-	+	+
Alkaloids	+	+	+	+	+	+	+	+
Quinonic substances	-	-	+	+	-	-	+	+
Anthraquinones	-	+	+	+	-	-	-	-
Saponin	+	-	+	-	+	-	+	-

(+) : Presence; (-) : Absence

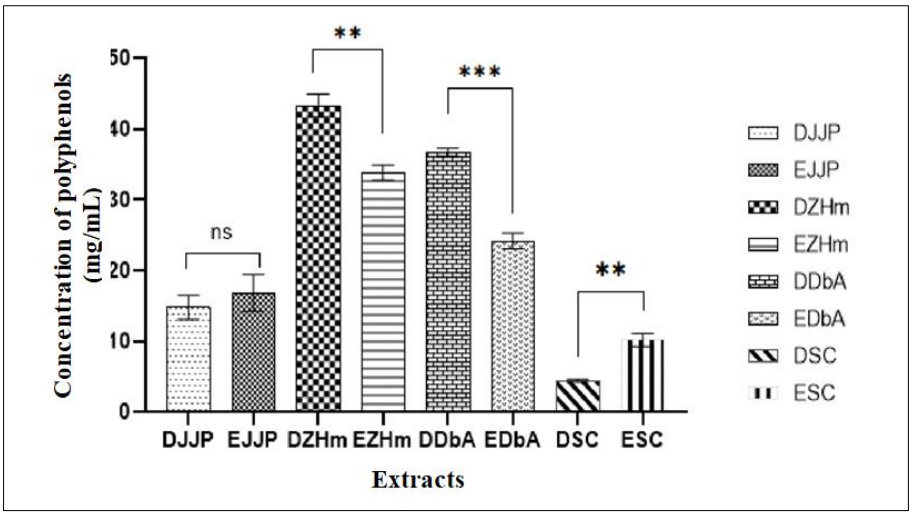

Figure 1-A: Total phenol contents of aqueous and hydroethanolic extracts of the recipes

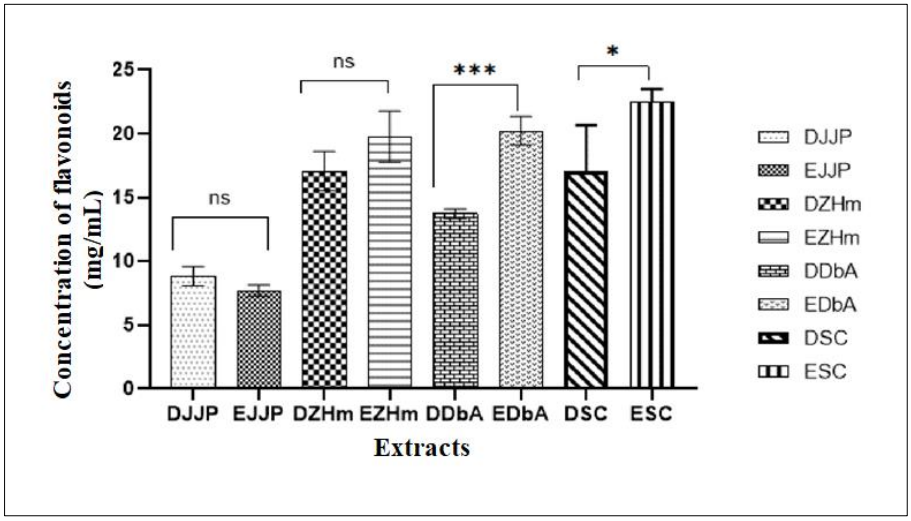

Figure 1-B: Total flavonoid contents of aqueous and hydroethanolic extracts of the recipes *flavonoid*

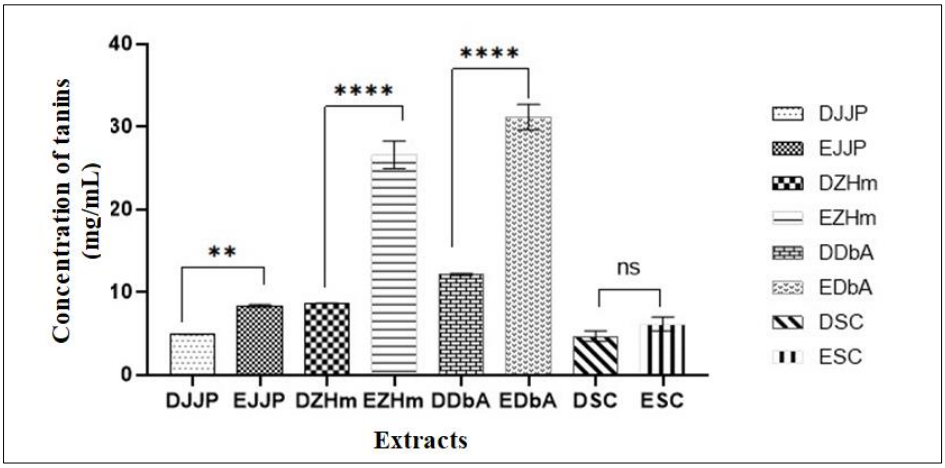

Figure 1-C: Total tanin contents of aqueous and hydroethanolic extracts of the recipes
Figure 1: Total phenolic compound contents of extracts from the four plant recipes

3.3.4. Total Alkaloid Content

The alkaloid concentrations obtained by studying the different plant recipes are shown in Table IV. Analysis of the results shows that alkaloid concentrations vary between plant recipes. The JJP recipe contains the most alkaloids (36.11% Atropine equivalent), followed by DbA (9.11% Atropine

equivalent), then ZHm (8.90% Atropine equivalent) and finally Sc (1.11% Atropine equivalent).

Table IV: Result of total alkaloid content

RECIPES				
	JJP	**ZHm**	**DbA**	**Sc**
Total alkaloid concentrations	36.11 %	8.90 %	9.11 %	1.11 %

3.4. Antioxidant Potential of the Extracts

3.4.1. Inhibitory Power of the DPPH Radical

Table V shows the results of the anti-free radical activities of different extracts from plant recipes compared with ascorbic acid. The table shows that all the recipes studied inhibit the DPPH radical to varying degrees. Anti-free radical activity ranged from 40.58% *(DSc*) to 82.49% (EDbA). The best inhibitory activities were progressively observed with DbA (82.35%; 82.49%), ZHm (78.68%; 75.85%) and ESc (77.44%) extracts. These extracts generated IC_{50} values of 80 µg/mL, 30 µg/mL and 10.67 µg/mL respectively for DJJP, DZHm and DDbA. Similarly, the IC_{50} values for the hydroethanolic extracts of recipes EJJP, EZHm, EDbA and ESc were 70 µg/mL, 20 µg/mL; 6.45 µg/mL and 35.20 µg/mL, respectively. Ascorbic acid had an IC50 = 6.05 µg/mL (Table 2). The inhibitory concentration of Sc decoctate (DSc) was not graphically indeterminate.

Table V: Results of the scavage plant extrac

Substances		Decocted				Hydroethanolic extracts			
	Ascorbic acid	JJP	ZHm	DbA	Sc	JJP	ZHm	DbA	Sc
% inhibition	99	69.65	78.68	82.35	40.58	70.05	75.85	82.49	77,44
IC_{50} (µg/mL)	6.05	80	30	10.67	-	70	10.67	5	35,20

3.4.2. Reducing Power of Ferric Ion to Ferrous Ion

The presence of reducing compounds in the extracts caused the Fe^{3+} ion (complexed) to be reduced to Fe^{2+} ion. The results obtained are summarised in Figure 2. The reducing power of iron is proportional to the increase in concentration of these four recipes. According to these results, the best activities were generated with the decoctions of the ZHm and DbA recipes. The optical densities obtained were 1.50, 1.69 and 0.93 at a concentration of 1 mg/mL for the DZHm, DDbA and DSc recipes respectively. The optical density of the DJJP recipe is 0.07 at a concentration of 1 mg/mL. Recipes (ZHm) and (DbA) show antioxidant activity similar to that of the reference molecule Butyl hydroxy toluene (BHT), whose OD is 1.28 at a concentration of 1 mg/mL. Compared with the decoctates from the JJP and Sc recipes, the decocts from the DbA and ZHm recipes were better at reducing iron at low concentrations.

Observation of Figure 2 shows that the hydroethanolic extracts from the EJJP, EZHm, EDbA and ESc recipes all reduced the Fe3+ ion (complexed) to the Fe2+ ion. At the maximum concentration of 1 mg/mL, the ODs were 0.71, 1.49, 1.80 and 1.40 for EJJP, EZHm, EDbA and ESc respectively. Recipes (ZHm) and (DbA) significantly (P<0.001) reduced iron compared to the recipe (JJP). The JJP, ZHm, DbA and Sc recipes are rich in phenolic compounds and exert variable antioxidant activities *in vitro*.

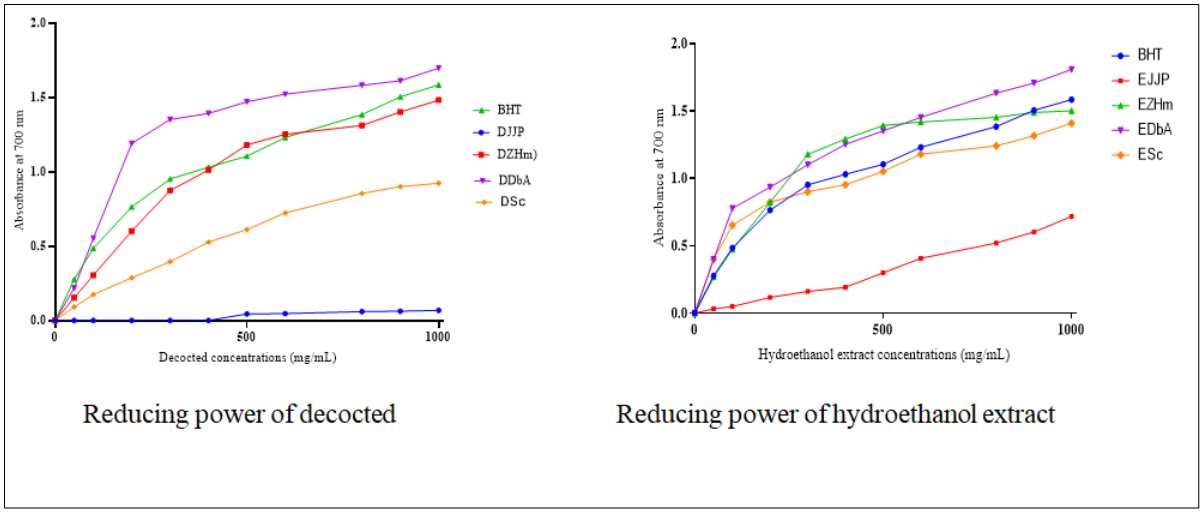

Figure 2: Reducing power of extracts from four recipes and BHT

3.5. Toxicological Studies

This study is used to determine the toxicological class of a substance and the doses that can be injected into animals without causing death. No deaths were recorded at the various doses of 300 and 2000 mg/kg body weight (bw) of aqueous extracts of ZHm, DbA and Sc during the two weeks of observation. Administration of 2000 mg/kg bw of hydroethanolic extract of ZHm (EZHm) caused behavioural disturbances such as somnolence and apathy, which were observed in female rats. These disturbances only lasted about 30 minutes. The treated animals returned to normal after 30 minutes. These animals were normal for up to 14 days after administration of the product. It can be deduced that the LD50 of the aqueous and hydroethanolic extracts of ZHm, DbA and Sc is greater than 2000 mg/kg Bw.

On the other hand, administration of the aqueous and hydroethanolic extracts of JJP at doses of 300 and 2000 mg/kg bw resulted in the death of the animals 3 h and 1 h respectively. This analysis shows that the extracts from the three recipes (ZHm, DbA and Sc) are not toxic by the oral route and belong to category 5 of the Globally Harmonised System of Classification (GHS). The JJP extracts, on the other hand, can be classified under category 1 of the GHS.

3.6. Effect of *Spathodea Campanulata* Extracts Sickling Inhibitory

Table VI summarises the effect of plant extracts and phenylalanine on the inhibition of falciformation of erythrocytes in the presence of sodium meta-bisulphite for two hours. All the substances tested (extracts and phenylalanine) inhibited the falciformation of HbSS erythrocytes in anaerobic media. Analysis of the results shows that after 2 h of contact with the extracts, the inhibition rate was 55.15%, 70% and 77.09% with the respective concentrations of 2.5, 5 and 10 mg/mL of ESc, while the decoctate (DSc) generated inhibition percentages of 51.33%, 61.66% and 72% at the same concentrations. Phenylalanine, which was used as a reference molecule, also inhibited the progression of falciformation in HbSS erythrocytes, generating inhibition rates of 60.16%, 68.33%. This inhibitory activity generated by the substances tested is dose-dependent, because as the concentration increases, the sickle cell rate decreases.

Table VI. DSC and ESC sickling inhibitory activity

Substances tested	Concentration: 2.5 mg/mL	Concentration: 5 mg/mL	Concentration: 10 mg/mL
ESC	55.15 %	70 %	77.09%
DSC	51.33%	61.66%	72%
Phenylalanine	60.16 %	68.33 %	83.65 %

4. DISCUSSION

Sickle cell anaemia is an inherited genetic disorder belonging to the haemoglobinopathy family. It results from a mutation in the 6th codon of the β-globin gene located on chromosome 11 [31]. This mutation substitutes valine for glutamic acid, leading to the synthesis of an abnormal haemoglobin called haemoglobin S (HbS) [32, 13]. In situations of hypoxia (absence of oxygen), acidosis (an abnormal decrease in blood pH) and high levels of 2-3 DPG, the solubility of haemoglobin S decreases and it polymerises by gelling, resulting in dehydration [33, 34]. This leads to the deformation of normal red blood cells into abnormal red blood cells. The sickle-shaped red blood cells are unable to pass through the small vessels (capillaries) and tend to become blocked, causing thrombosis and ischaemia in the organs [35, 36]. This falciformation induces an increase in haemolysis leading to the high production of reactive oxygen species (ROS) through the release of haem in the vessels [37]. This is followed by the development of an inflammatory response characterised in particular by activation of the vascular endothelium, which then produces various molecules including vascular adhesion molecules [38, 39]. This inflammatory response leads to a reduction in the lumen of blood vessels and therefore to the blockage of sickle cell red blood cells, causing vascular occlusions which are a source of morbidity and mortality [40, 41]. Sickle cell disease involves several organs during crises, so appropriate therapeutic strategies need to be defined for its management in order to preserve vital organs. This leads us to determine the harmful and antioxidant properties of extracts from ZHm, DbA, JJP and Sc recipes used in traditional settings.

Analysis of the results of the acute toxicity tests revealed that the decocted and hydroethanolic extract from the ZHm, DbA and Sc recipes are not toxic by the oral route. In fact, oral administration of the extracts from the ZHm, DbA and Sc recipes did not cause any signs of toxicity during the study. This analysis shows that the three recipes belong to category 5 and are considered to be non-toxic by the oral route at doses of 300 and 2000 mg/kg bw. These results are in line with the research work of Kplé [42], and koulai [43]. These authors showed that these plant recipes were not toxic at the doses tested orally in rats. Contrary to what was observed with the ZHm, DbA and Sc recipes, the JJP recipe generated toxicity in rats. Administration of the extracts at doses of 300 and 2000 mg/kg bw caused the death of the rats in less than 24 hours. This toxicity is thought to be linked to the presence of apigenin, latex, 12-deoxy-16-hydroxylphorbol and toxalbumin in the leaves of *J. gossypiifolia*.

This assertion was affirmed by Nagaharika [44], Magili and Bwatanglang [45], and Wu [46]. In addition, the work of Ilic [47], revealed that the aqueous extract of *A. melegueta* caused lesions in the liver after 28 days of treatment of the animals. In addition, the alkaloid content of this recipe could contribute to its toxicity. It is therefore likely that the presence of considerable quantities of alkaloids in the fruits of *A. melegueta* and components of the leaves of *J. gossypiifolia* acted in synergy to cause the toxicity of the JJP recipe. Although the World Health Organisation (WHO) now advocates the use of drug combinations in the treatment of diseases [48], toxicological studies should be carried out before their widespread use, as the efficacy of a substance in pharmacology is not sufficient to justify its possible introduction in therapeutics.

Chemical screening identified the presence of primary metabolites (aromatic amino acids) and secondary metabolites (phenolic compounds, saponins, alkaloids, free quinones, steroids and terpenes) in the plant recipes studied as a whole. On the other hand, aromatic amino acids, leuco-anthocyanins and anthraquinones were absent from *Spathodea campanulata* extracts. Gallic tanins and quinone substances were not found in extracts from JJP and DbA recipes. These data corroborate previous work by Akakpo-Akue [49], on the presence of chemical compounds such as alkaloids, sterols/terpenes, saponins, flavonoids and tanins in these plant recipes used in the treatment of sickle cell disease in CI. On the other hand, the results of Jayanthi [50]. Devanand [51], and Duryat [52], show the presence of flavonoids, alkaloids and amino acids by LC-MS GS-MS analysis methods on the ethanolic extracts of *S. campanulata* flowers. This difference is thought to be due to the place of harvest and the organ used. These results prove that these plants contain active ingredients and could justify their use in traditional African pharmacopoeia.

The *in vitro* antioxidant evaluation showed that the extracts from the plant recipes are capable of reducing DPPH free radicals and the ferric ion (fe^{3+}). These two extracts from the recipes generate anti-free radical and antioxidant activities that vary from one recipe to another and, for the same recipe, from one extract to another. A comparison of IC_{50} values shows that hydroethanolic extracts (EDbA >EZHm>ESc>EJJP) inhibit the DPPH radical and reduce ferric ion more than decocteds (DDbA >DZHm>DSc>DJJP) from one recipe to another. These antifree radical and antioxidant properties are thought to be based on the phenolic compound contents of the recipe extracts. In addition, quantification of flavonoids known for their antioxidant power showed that both extracts had high accumulations of polyphenols and total flavonoids. However, the hydroethanolic extracts (EDbA >EZHm) were richer in total flavonoids than the decocted extracts (DDbA >DZHm>DSc>DJJP), which corroborates the antioxidant activity results obtained by the DPPH tests and the reducing power of fe^{3+}.

This could be explained by the extraction solvent, the extraction method and the boiling temperature, which would have affected the extraction of flavonoids, the molecules responsible for antioxidant activity. Results similar to ours mention the inhibition of DPPH radicals and ferric ions in extracts from polar solvents [53, 54]. According to the work carried out by Mahmoudi [55], maceration would be preferable for extracting flavonoids compared to decoction in his study on Artichoke (cynara scolymus l.) flower. Furthermore, Bohui [56], demonstrated that the extraction time could lead to the degradation of certain natural substances such as polyphenols and flavonoids. Numerous studies have established a correlation between antioxidant activity and phenolic compound (flavonoid) contents. The ability of these molecules to trap free radicals depends essentially on their structure. Indeed, the most active flavonoids are those containing 3'4'dihydroxy groups on the B ring and/or a 3OH group on the C ring [57]. Polyphenols seem to be efficient hydrogen donors to the DPPH radical, due to their ideal structural chemistry, without however neglecting other minor phenolic compounds, because synergy between different chemicals should be considered in biological activity [57].

With regard to the antisickling activity of the DSc and ESc extracts, the results show that all the concentrations tested inhibited the falciformation of HbSS erythrocytes in anaerobic environments in the same way as phenylalanine (reference molecule).These results, therefore, show that phenylalanine and SC extracts are capable of attenuating the deleterious effects generated by sodium metabisulphite. The percentages of ESc falciformation inhibition were 55.15%, 70% and 77.09% respectively for concentrations of 2.5, 5 and 10 mg/mL, while those for decocted (DSc) were 51.33%, 61.66% and 72% at the same respective concentrations. Comparison of the inhibitory activities of the two *Spathodea campanulata* extracts shows that this plant has the capacity to inhibit falciformation.

The results obtained with the bark of the trunk of *S. campanulata* are superior to those obtained with the flowers of the said plant in the work carried out by Bamimore and Elujoba [58], on the inhibition of falciformation. Indeed, the results of their work show that the inhibition of falciformation was between 10% and 37.92% respectively at concentrations between 2 and 10 mg/mL, whereas those obtained in this work are between 51.33% and 72% respectively at concentrations between 2.5 and 10 mg/mL. This can be explained by the different organs of *S. campanulata* used in these studies. Barks and leaves are generally rich in secondary metabolites as these are known to be one of the main sites of secondary metabolite synthesis in plants [59].

According to Kambale [60], SS red blood cells contain a relatively higher proportion of methaemoglobin than normal red blood cells. We know that increasing the proportion of methaemoglobin reduces the affinity of haemoglobin for oxygen, as iron in its oxidised state is unable to bind this molecule which is essential for life. Oxidation of haemoglobin (Fe2+) to methaemoglobin (Fe3+) is due to the significant oxidative stress in sickle cells. A plant that reduces the ferric ion (Fe3+) would therefore have an antioxidant effect on sickle cell red blood cells. The results of this study confirm the antisickling activity of Sc extracts, as well as the reduction of the DPPH radical and ferric ion *in vitro*. Studies carried out on JJP, DbA and ZHm recipes by Kplé [61], Akakpo-Akue [62], and Akakpo-Akue[13], respectively show that these recipes are rich in primary and secondary metabolites and generate remarkable antisickling activity compared with the results obtained with SC extracts.

The good performance of these plant recipes is thought to be linked to their richness in phenolic compounds and aromatic amino acids, and to the synergistic or additive effect of molecules with antisickling activity derived from the different plants present in these recipes. However, the toxicity of poly-specific recipes should be studied before they are used.

5. CONCLUSION

In this work, we set ourselves the objective of determining the safety, antioxidant and antisickling activities of a number of anti-sickle cell recipes in order to contribute to the management of sickle cell disease in Côte d'Ivoire.

From a phytochemical point of view, these plant recipes are rich in phenolic compounds, alkaloids, steroids and terpenoids. The antioxidant activity of hydroethanolic extracts is greater than that of decocteds. These recipes have anti-sickle cell properties *in vitro*. The use of these anti-sickle cell recipes in traditional environments is therefore justified. However, not all of them were tolerant, and one of them (JJP) was toxic at a dose of 300 mg/kg bw.

It is therefore important to study the safety of the recipes proposed by our naturotherapists before consumption. It is always possible to optimise these recipes with a view to better management.

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