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Effects of Methanolic and Aqueous Extracts of *Griffonia simplicifolia* (Fabaceae) on the Inhibition of Falciformation of Human Hb SS Erythrocytes

Joel Akakpo-Akue[1], Gnamien Marcel Ahon[1,2], Tatiana Kangah Mireille Kplé[1*], Loukou Marie Louise Djaha[1], Fofie Yvette[3], Sanogo Ibrahime[4], Bahi Calixte[1], Djaman Allico Joseph[1,5]

[1]Laboratory of Biology and Health, UFR Biosciences, Université Félix Houphouët-Boigny (UFHB), Abidjan, Côte d'Ivoire
[2]National Pedagogical Institute of Technical and Professional Education, Abidjan, Côte d'Ivoire
[3]Pharmacognosy Laboratory, UFR of Pharmaceutical and Biological Sciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire
[4]Department of Clinical Haematology, University Hospital of Yopougon, Abidjan, Côte d'Ivoire
[5]Institut Pasteur de Côte d'Ivoire, Abidjan, Côte d'Ivoire
Email: *tatianakangah@gmail.com

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Abstract

Sickle cell disease (SCD) is a genetic blood disorder that affects the shape and transportation of red blood cells (RBCs) in blood vessels, leading to various clinical complications. Sickle cell disease is a widespread genetic disease in Black Africa. The objective of this study was to evaluate the sickling inhibition activity of *Griffonia simplicifolia.* Quantitative and qualitative tests were used to determine the major groups of secondary metabolites present in the *Griffonia simplicifolia* leaves extracts and the modified Emmel test was used to perform the study of sickling inhibition activity. The OECD 423 toxicity study showed that at a single dose of 2000 mg/kg bw, *Griffonia simplicifolia* leaves extracts are not toxic. All tested substances inhibit erythrocyte falciformation in a dose-dependent manner. The percentages of inhibition were 50.35%, 73% and 94.23% for aqueous extract, hydromethanolic extract and phenylalanine respectively at the concentration of 15 mg/mL. The methanolic extract (70%) had higher activity compared to the aqueous extract. These results suggest that the *Griffonia simplicifolia* extracts have some potential to be used as alternative antisickling therapy in SCD management.

Keywords

Griffonia simplicifolia, Antisickling, Aqueous Extract, Methanolic Extract, SS Erythrocyte

1. Introduction

To deal with health problems, 80% of the world's population relies on traditional medicine and remedies from traditional pharmacopoeia [1]. The use of herbal medicines is widespread and of increasing health and economic importance. Herbal remedies are sought for the treatment of several pathologies, even those known as genetic [2]. Sickle cell disease is an autosomal recessive genetic disorder characterized by its high frequency [3]. The disease results from a point mutation in the sixth codon of the β globin gene located on chromosome 11. This mutation will result in the replacement of glutamic acid with valine producing an abnormal hemoglobin called hemoglobin S. Under hypoxia, Hb S hemoglobin polymerizes. The polymers in the form of tubular fibers give the red blood cell the sickle shape characteristic of the disease. The falciformation of erythrocytes in a patient is the origin of vaso-occlusive crises, susceptibility to infections and hemolytic anemia [4]. According to the WHO, 500,000 sickle cell children are born each year in the world, 30% of these children are in Africa [5]. Moreover, sickle cell disease is the most common hemoglobinopathy in black Africa, where its prevalence, increases from 16% to 40%, depending on the region [6]. Sickle cell disease is the cause of 5% of child deaths on the African continent [7]. In Côte d'Ivoire, 6000 to 8000 births of children with sickle cell disease are recorded per year, of which 40% die before the age of five (5) years [8]. Sickle cell disease is therefore a public health problem in Côte d'Ivoire.

The management of the condition is lifelong treatment [9]. The therapeutic strategy includes preventive treatments against infections, analgesic treatments for pain and blood transfusions. Hematopoietic stem cell transplantation gives insufficient results, due to the rejection phenomenon and also difficulties in finding suitable donors. However, there is great hope in gene therapy [10]. All these biotechnologies require specialized infrastructures and qualified staff. As to the symptomatic treatments, they are difficult to access for African populations with low-income. Thus, herbal medicine could present an alternative that could offer adequate treatment to sickle cell subjects in our countries [11]. Indeed, medicinal plants contain a multitude of primary and secondary metabolites with diverse biological activities that can deal with sickle cell syndrome which has variability in expression. Some works done on medicinal plants have highlighted the chemical composition of plants and their inhibitory activities [12], antifalcemic [13] [14]; also on reversal sickle cell morphology activity [15].

In this perspective an ethnobotanical survey was conducted by [16]. The aim of this survey was to identify some anti-sickle cell plants. *Griffonia simplicifolia* which is the subject of our study is one of those. This species has been the subject of several studies. Indeed, 5-hydroxytryptophan (5-HTP) has been identified as the most important chemical element of this plant species. Also secondary metabolites such as tannins, alkaloids, flavonoids, saponins and coumarins have been identified in the methanoic, aqueous and hydro-methanoic extract of its seeds [17] [18] [19]. Other researchers have studied its antioxidant activity [19].

The objective of the present study was to assess the sickling inhibitory activity of *Griffonia simplicifolia* leaves.

2. Material and Methods

2.1. Biological Materials

2.1.1. 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) of Abidjan. They were used to study the acute oral toxicity of the aqueous extract and the hydro-methanolic extract of *Griffonia simplicifolia* leaves.

2.1.2. Human Material

The blood sample used to evaluate the antifalcemic activity of *Griffonia simplicifolia* leaves were collected from sickle cell patients with HbSS genotype confirmed by electrophoretic test. Then, the samples were collected in a tube (EDTA) and stored at 4˚C in a refrigerator for further determination of the antifalcemic activity.

Inclusion criteria

To be included in this study, patients should have been sickle cell patients of SS genotype, whose blood was not drawn or transfused during the two months prior to blood sampling. The sampling of volunteers did not take into account age or gender.

Exclusion Criteria

Any patient who did not have hemoglobin SS was excluded from our tests.

ETHIC

For this study, informed consent was obtained from the volunteers.

2.1.3. Plant Material and Preparation of Extracts

Fresh leaves of *Griffonia simplicifolia* (Fabaceae) were collected in April 2022 in Didievi in the central-eastern part of Côte d'Ivoire. After harvesting, a sample of this plant was identified at the Centre National Floristique of the Université Félix Houphouët Boigny de Cocody under the number UCJ009411.

✓ **Total aqueous extract**

It *was* obtained by the maceration method described by [20]. One hundred grams (100 g) of *Griffonia simplicifolia* powder previously obtained was dissolved in one liter of distilled water. The mixture was then homogenized 10 times in the row using a Severin brand blender. The homogenate obtained was wrung out in a cotton fabric square and then filtered three times on cotton wool and once on filter paper (3 mm). The filtrate was evaporated at 50˚C using an oven (VENTICELL 55).

✓ **Hydro-methanolic extract**

Using [21] the method of, 50 g of *Griffonia simplicifolia* leaves powder were cold stirred up in 1.5 L of 70% methanol for 48 h on a magnetic stirrer. The

mixture was successively filtered through cotton wool and WATHMAN paper (3 mm). The residue was removed and the brownish alcoholic solution was evaporated at reduced pressure in BUCHI rota-vapor at 30˚C for 24 h.

2.1.4. Phytochemical Screening

The phytochemical screening, a qualitative test, reveals he presence of the main chemical groups such as sterols and polyterpenes, alkaloids, tannins, polyphenols, flavonoids, quinones and saponins. The references and developers used are respectively: Liebermann's reaction for sterols and polyterpenes, Burchard's reagent (iodine-iodide reagent) and Dragendorff's reagent (potassium iodo-bismuthate reagent) for alkaloids, Stiasny's reagent for tannins, ferric chloride (FeCl3) reaction for polyphenols, cyanidine reaction for flavonoids, Bornstraegen's reagent for quinones and for saponins the foam index was determined [22].

2.2. Phenolic Compound Contents

✓ **Total Phenol Content**

The total phenolics content was determinated using Folin–Ciocalteu assay method [23]. The absorption was read at 745 nm against a blank (the spectrophotometer Jenway 7315, by bibby scientific United Kingdom). A standard range based on of a gallic acid stock solution (0.1 mg/mL) under the same conditions as the assay permited to determine the amount of phenols in the sample (mg GAE/g) by using the equation: $y = 10.12x$.

✓ **Flavonoids Content**

The flavonoid content was determined from the calibration curve made of a 0.01 mg/mL stock solution of quercetin, using direct quantification by aluminium chloride method [24]. A standard range established from a quercetin stock solution (0.1 mg/mL) under the same conditions as the assay was used to determine the amount of flavonoids in the sample by using the equation: $y = 13.70x$.

✓ **Tannin Content**

The determination of tannin content was performed according to the method described by [25].

One (1) mL of methanolic extract was introduced into a test tube. To the contents of the tube was added 5 mL of vanillin reagent. The tube was allowed to stand for 30 min in the dark and the optical density (OD) was read at 500 nm against a blank. The amount of tannins in the samples was determined using a standard range established from a stock solution of tannic acid (2 mg/mL) under the same conditions as the test. The tannin content was obtained from the equation: $y = 3.92x$.

2.3. Acute Toxicity

The oral acute toxicity of *Griffonia simplicifolia leaves* extract was performed according to OCDE protocols [26]. The test was performed on 9 rats divided into 3 groups of 3 animals, comprising one control and one test group. These animals fasted 15 hours then weighed and treated by esophageal gavage. Group 1;

control group received aqueous. Group II and III received 2000 mg/Kg b.w hydro-methanolic and total aqueous extracts respectively. Upon treatment, these animals fasted for additional 3 hours. During this period, signs of toxicity including sensitivity to pain, noise, tail state, stool appearance and mobility and death were noted.

2.4. Sickling Inhibitory Activity

The test was performed according to Emmel test which was slightly modified [27] [28]. Confirmed SS Blood was washed for five minutes at 3000 rpm three times in a row to remove the supernatant. Fifty (50) µL of washed blood was mixed to 50 µL of sodium metabisulfite 2% solution and 50 µL of different plant extracts (0.625, 1.25, 2.5, 5, 10 and 15 mg/ml). After 120 minutes of incubation, morphological analysis was carried out using Huma SCOPE Advanced optical microscope and the residual percentage of sickle cells was determined. The same concentrations were used for phenylalanine and tested under the same conditions as the extracts. The sickling inhibitory activity was expressed in percentage of sickle cells formed in the presence of the plant extracts compared to the number of sickle cells present in the negative control. This activity was determined by the formula noted below:

$$AA = (P0 - P1)/P0 * 100 \quad (1)$$

AA: antisickling activity; P0: sickle cells rate in the control; P1: sickle cells rate in test tubes.

2.5. Statistical Analysis

Statistical analyses of the experimental results were performed using GraphPad Prism 7 software (Microsoft, USA). Values were presented as mean ± standard error on the mean. Tukey's multiple comparison test at the 5% level was applied to assess the significance of the observed differences.

3. Results and Discussion

3.1. Extraction Efficiency

The extractions of *Griffonia simplicifolia* leaves yielded 12.02% and 14.50% dry extract respectively for aqueous and methanolic (70%) extraction for 100g of powder in one liter (1 L) of water and 50 g of powder in 1.5 L of methanol (70%).

3.2. Phytochemical Study

The qualitative phytochemical study carried out on the different extracts of *Griffonia simplicifolia* leaves revealed the presence of several families of bioactive compounds. These results have been recorded in Table 1. They show that these extracts contain gall tannins, steroids, alkaloids and triterpenes. On the other hand, polyphenols, flavonoids and gall tannins are absent in the aqueous extract but present in the methanolic extract (70%).

Table 1. Qualitative study of the chemical compounds present in the extracts of the leaves of *G. simplicifilia*.

	Chemical constituents								
	Stérols/ Polyterpene	Polyphenols	Flavonoide	Tannins		Quinones	Alcaloids		Saponins
				Catholic	Gallic				
EXTRACTS									
Aqueous extract	+	−	−	−	−	−	+	+	+
Methanolic extract 70%.	+	+	−	+	−	+	+	+	+

3.3. Phenolic Compound Contents

The results of the quantitative study of *Griffonia simplicifolia* leaves extracts were recorded in Table 2. The tannins content of the aqueous extract of *Griffonia simplicifolia* leaves is 14.49 ± 0.2 mgEcat/mg while, that of the hydro-methanolic extract is 14.39 ± 0.3 mgEcat/mg. The flavonoid contents were measured from the standard equation of $y = 13.70x$ curve. The aqueous extract gave the value of 10.66 ± 0.3 mgEQ/g and that of methanolic (70%) gave 10.08 ± 0.8 mgEQ/g. As for the polyphenol content, it was obtained from the equation $y = 10.12x$. The aqueous extract gives a value of 8 ± 0.2 mgEGA/g and that of methanolic extract is 14 ± 0.6 mgEGA/g.

3.4. Toxicological Studies

Rats treated with the hydro-methanolic and aqueous extracts of *Griffonia simplicifolia* leaves showed behavior change including decreased in sensitivity to pain, noise and locomotion. No death was noticed within the treated animals. The oral LD50 of *Griffonia simplicifolia* leaves extracts is therefore greater than 2000 mg/Kg b.w.

3.5. Effect of *G. simplicifolia* Extracts on the Inhibition of Falciformation of Erythrocytes

Figure 1 summarizes the effect of plant extracts and phenylalanine on the inhibition of falciformation of erythrocytes in the presence of sodium meta-bisulfite for two hours. The analysis of the results shows that after 2h of contact with the extracts, the inhibition rate was:

- 16.33%, 22.66% and 39% for the concentration of (0.625 mg/mL) for aqueous extract, hydro methanolic extract and phenylalanine respectively.
- 22.66%, 32.33% and 49% for the concentration of (1.25 mg/mL) for aqueous extract, hydro methanolic extract and phenylalanine respectively.
- 25% 41% and 59.66% for the concentration of (2.5 mg/mL) for aqueous extract, hydro methanolic extract and phenylalanine respectively.
- 30%, 55.34% and 68% for the concentration of (5 mg/mL) for aqueous extract, hydro methanolic extract and phenylalanine respectively.
- 39%, 64.33% and 82.68% for the concentration of (10 mg/mL for aqueous extract, hydro methanolic extract and phenylalanine respectively.

Table 2. Quantitative study of the chemical compounds present in the extracts of the leaves of *G. simplicifilia*.

Parameters plant extracts	Yield (%)	total Polyphénols (**mgEGA/g**)	Total Flavonoïdes (**mgEQ/g**)	Total Tanins (**mgECat/g**)
Aqueous extract	12.02	8 ± 0.2	5.66 ± 4.8	10.89 ± 0.2
Methanolic extract 70%.	14.50	14 ± 0.6	10.08 ± 0.1	14.39 ± 0.3

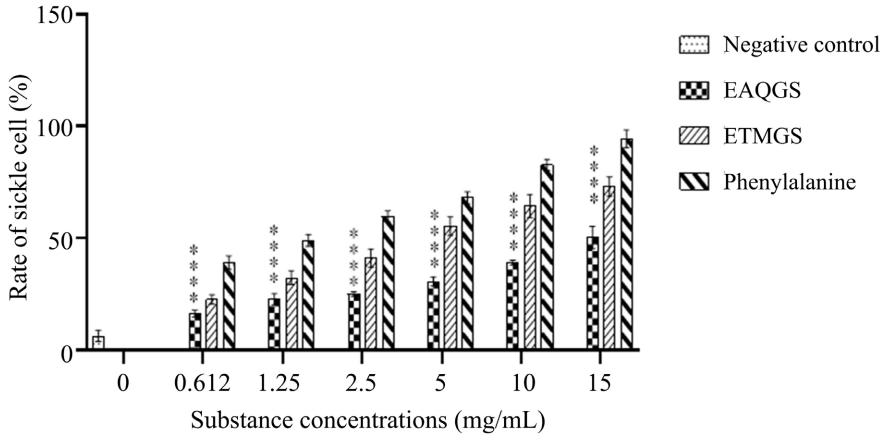

Figure 1. Effect of test substances on the evolution of the sickle cell rate in the presence of sodium meta-bisulfite after 120 min of contact. Test drugs: significant from normal control, ****P < 0.0001; Mean ± S.E.M = Mean values ± Standard error of means of three experiments.

- 50.35%, 73% and 94.23% for the concentration of (15 mg/mL) for aqueous extract, hydro-methanolic extract and phenylalanine respectively.

This inhibitory activity is dose dependent because the concentration increases the sickle cell rate decreases.

4. Discussion

This work involved the investigation of some chemical groups, determination of acute toxicity and evaluation of the antisickling activity of aqueous and hydro-methanolic extracts of *Griffonia simplicifolia* leaves.

Phytochemical analysis of *Griffonia simplicifolia* extracts revealed the presence of secondary metabolites which are: alkaloids, catechic tannins, quinones, sterols, terpenes and saponins. Flavonoids and polyphenols were not present in the aqueous extract but present in the hydro-methanolic extract. Studies have shown the benefits of these compounds found in *Griffonia simplicifolia* leaves. Alkaloids and polyphenols have antioxidant properties. According to Akhila *et al.* [29] the presence of saponosides, triterpenes or sterols in a plant extract would participate in the anti-inflammatory, bactericidal and antimicrobial activities of these plants.

Our results are in agreement with those of Guirlo and Offoumou *et al.* [16] [17] who showed that the methanolic extract of *Griffonia simplicifolia* bark contained the alkaloids, tannins, saponins and terpenoids. On the other hand, they differ from those obtained by Nyarko *et al.* [18]. These showed in their work the

absence of saponins and the presence of glycosides, tannins, flavonoids, in the characterization tests of polyphenols, flavonoids, saponin and coumarins in aqueous, ethyl acetate, methanolic and hydro-methanolic extracts of *Griffonia simplicifolia*. This difference could be due to the place of collection, drying conditions and extraction solvents.

The results obtained from the phytochemical study carried out on the aqueous and hydro methanolic extracts allowed to determine the content of polyphenols, flavonoids and tannins. These results show that *Griffonia simplicifolia* extracts contain varying concentrations of polyphenols (8 and 14 mgEGA/g), flavonoids (5.66 and 10.08 mgEQ/g) and tannins (10.89 and 14.39 mgECat/g) in aqueous and hydro-methanolic extracts respectively. This difference in content in the two extracts could be explained by the difference in the solvents of the two extracts. These same secondary metabolites were highlighted by Offoumou *et al.* and Nyarko *et al.* [17] [18].

Regarding the acute toxicity study, it provided information on the LD50, therapeutic index and the degree of safety of a pharmacological substance [30]. The results show that single dose oral administration of 2000 mg/kg bw of the aqueous and hydro-methanolic extracts of *Griffonia simplicifolia* to the rats did not exert any toxic effect during the 24 hours of observation. Also, no mortality was reported during the 14-day observation period. According to OECD Test Guideline 423 for chemical testing, aqueous and hydro-methanol extracts of *Griffonia simplicifolia* have a lethal dose 50 (LD50) greater than 2000 mg/kg bw. Based on this guideline, *Griffonia simplicifolia* extracts can be classified as GHS hazard category 5. This would suggest that the single dose of 2000 mg/kg bw of *Griffonia simplicifolia* leaves extracts would not influence the physiology and metabolism of rats. Our results are in line with Bidie *et al.* [20], who showed that the hydroethanol extract of *Griffonia simplicifolia at* the dose of 2500 mg/kg bw, did not generate any signs of toxicity in rats. In view of the results obtained, *Griffonia simplicifolia* extracts contain bioactive metabolites that could improve health.

Under the conditions of hypoxia, the aqueous and hydro-methanolic extracts of the plant inhibited the falciformation of red blood cells Hb SS genotype. The inhibition rate were for aqueous extract 16.33, 22.66%, 25% 30%; 39% and 50.35%, for hydromethanolic extract 22.66%; 32.33%; 41%; 55.34%; 64.33% and 73% and finally 39%; 49%; 59.66%; 68%; 82.68% and 94.23% for phenylalanine for the concentrations ranging from 0.625 to 15 mg/mL. All the extracts were found to be active on the inhibition of falciformation.

The activity of *Griffonia simplicifolia* leaf extracts is dose dependent, the higher the concentration, the higher the antifalcemic activity of the extracts. This antisickling activity is increasing according to the time of contact. It appears from this analysis that the hydro-methanolic extract has a better antisickling activity than the aqueous extract. This antisickling activity of the hydro-methanolic extract could be explained by the fact that the hydro-methanolic extract is the

solvent that would better concentrate the compounds responsible for the anti-falcemic activity. These compounds responsible for the antisickling activity are polar in nature [8] [13], obtained similar results with polar compounds with antisickling activity. Moreover according to Mpiana *et al.* [31], nitrogenous compounds such as phenylalanine are involved in the inhibition of deoxygenated Hb S polymerization and to the reversal of falciformation. Thus, the presence of alkaloids could participate in the antisickling activity of the extracts. The presence of these molecules in these extracts would induce erythrocyte membrane stability, reduce HbS polymerization [32]. In addition, these molecules could increase the affinity of oxygen for hemoglobin and would promote better water supply in the erythrocyte [5] [33]. These compounds would prevent the peroxidation of membrane lipids and thus prevent erythrocyte lysis.

From all the above, we can say that, *Griffonia simplicifolia* extracts generate an antisickling activity at all concentrations tested. We can therefore say that the use of this plant for the treatment of sickle cell disease in traditional environment is justified.

5. Conclusion

The results obtained revealed that the extracts are not toxic and have a potential to inhibit the antisickling. It appears from these results that the hydro methanolic extract has a better activity than the aqueous extract. This difference could be related to the content of phenolic compounds in the hydro methanolic extract. However, further toxicity and phytochemistry studies are needed.

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Authors' Contributions

All authors participated in the completion of the work and then read and approved the final manuscript.

Consent (Where Ever Applicable)

An agreement was obtained from the ethic committee and an informed consent approved by each patient wishing to participate to the study.

Conflicts of Interest

Authors have declared that no competing interests exist.

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