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Chemical screening and antifalcemic evaluation of *Cajanus cajan* L. (Fabaceae), a species from the Congo

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ABSTRACT

Sickle cell disease is a genetic disorder that is a public health problem. Given the high cost of treatment, patients turn to traditional medicine with the use of plants, including *Cajanus cajan* L. (pois d'angole).

Objective: The objective of this work was to carry out a phytochemical study and to evaluate *in vitro* the antifalcemic effect of *C. cajan* seeds.

Methodology and Results: This experimental study was carried out in two phases: one devoted to the characterization of the seeds and the other concerned the study of the antifalcemic activity *in vitro* of these seeds. The study of antifalcemic activity was carried out on blood samples from 12 homozygous sickle cell patients aged between 4 and 22 years. The addition of plant extracts to the collected blood made it possible to evaluate the antifalcemic activity by detecting sickle cells under a light microscope. *C. cajan* seeds contain sterols, polyterpenes, polyphenols, flavonoids, tannins and alkaloids. The aqueous, methanolic and dichloromethane extracts decreased sickle cell counts by about 30 minutes of contact.

Conclusion and Application of Results: It appears from this study that *C. cajan* is cited as one of the plants used against sickle cell anaemia. This plant has shown antifalcemic activity in vitro, thus confirming the information obtained in traditional medicine and in the literature. This plant could therefore be used as food to relieve those who suffer from this chronic disease. This study showed that *Cajanus cajan* seeds have antifalcemic properties.

Keywords: *Cajanus cajan*, phytochemical screening, anti-sickle cell disease, sickle cell anaemia.

INTRODUCTION

Sickle cell disease in the world, particularly in Africa and the Congo, is a major public health concern. It is a genetic disorder of haemoglobin that was first described in the 1900s by a Chicago doctor, who was examining a student from the Caribbean island of Grenada with symptoms: cough, shortness of breath, fever, dizziness, headache, palpitations. Biology showed anaemia and blood smear of red blood cells (Girot, 2003). In 1917, it was EMMEL who demonstrated that the sickling of red blood cells only occurs when they are hypoxia, i.e. deprived of oxygen. This discovery led to the development of a screening test: the induced sickle cell test (EMMEL test). Sickle cell anaemia is a real public health issue, especially in developing countries, where the number of patients is large and where there is a lack of financial means to treat them. From a therapeutic point of view, only bone marrow transplantation currently provides satisfactory corrections for sickle cell patients (Pawliuk, 2001; Misaki, 2008). However, this highly specialized and expensive therapeutic approach is not available to most low-income patients such as those in Africa. The therapies currently proposed, including blood transfusion, the use of desferrioxamine and hydroxurea, provide only temporary solutions with an increased risk of contamination by viral or bacterial infectious agents and cytotoxicity (Ould, 2006; Lippi, 2010). For example, there is currently a renewed interest in herbal medicines in the treatment of sickle cell anaemia. Indeed, herbal medicine is the only alternative capable of offering relief to sickle cell patients. Several experimental proofs highlight the antisickle cell activity *in vitro* and *in vivo* of plants (Wambebe, 2001). In the present work, we report the results of the evaluation of the antifalcemic and *in vitro* effects of *C. Cajan* seeds. *C. cajan,* also known as "pigeon pea", is used as a food crop in tropical and subtropical regions of the world, which is a great source of protein, vitamin B and minerals for humans as well as animals and has a great contribution on medicinal uses (Proma Roy Orni, 2018). *C. cajan* is also known to be an excellent source of essential amino acids (lysine, phenylalanine, valine, leucine and isoleucine). The grains are also rich in fatty acids, the main ones being linoleic acid and palmitic acid (Fossou, 2012). This plant is very common. First, it is a food plant. Originally, from India, it was later exported and is currently found in the Congo in the sub-tropical climate zone. Relatively few studies have been conducted on *C. cajan.* This makes it difficult to accurately identify the components responsible solely for sickle cell activity. The components mentioned below are present in the seeds or leaves of *C. cajan* but not all of them have necessarily demonstrated anti-sickle cell activity. Interest in *C. cajan* for its sickle cell properties is fairly recent. It dates back to the work of Ekeke (1985) who found that a boiled extract of *C. cajan* seeds provided relief to sickle cell patients. The choice of this plant is due to its traditional and medicinal use. The aim of this study is to verify the hypothesis that the species grown in the Republic of Congo contains the active ingredient that would confer an anti-sickle cell activity.

MATERIALS AND METHODS

The study was conducted from February 14 to July 30, 2015. It is a single-center, prospective study that took place over a period of five and a half months at the National Center for Sickle Cell Disease (CNRD), located in Brazzaville, Republic of Congo. It is an establishment specialized in research, screening, care, follow-up and medical and psychological support for patients with sickle cell disease. Informed and written consent by each of the patients was obtained prior to the patients' blood draw. The protocol used has been

approved by the CNRD's haematology laboratory technicians.

Harvest site and plant material: The *C. cajan* pods used in this study were collected in August 20014 in Madingou, a locality located in southern Congo. Figures 1 and 2 show photographic images of *C. cajan* pods and seeds, respectively.

Fig.1: *Cajanus cajan* pods

Fig.2: *Cajanus cajan* seeds

Biological material : Heparinized blood samples used to assess antifalcemia activity were obtained at the CNRD Haematology Laboratory. None of these patients have recently been transfused**.** In order to confirm their homozygous SS nature, the blood samples were first characterized by paper-based electrophoresis using cellulose acetate gel at pH 8.5. Once the SS nature of the blood sample was confirmed, it was kept in a refrigerator at a temperature of 4°C.

Preparation of extracts: One (1) kg of shelled husks from the pods were dried at room temperature in the laboratory and reduced to fine powders using a scientific mill. Then, 10 g of powder was macerated several times in water, in methanol and dichloromethane (200 ml x1) respectively for 48 hours. The different fractions were filtered and the solvent was evaporated under reduced pressure using a rotary evaporator. The collected extracts are kept for later use.

Phytochemical studies: The search for groups of chemical compounds in the extracts was carried out in tubes and on Thin Layer Chromatography (TLC) according to the analytical techniques described in the literature (Wagner, 1987; Bekro, 2007).

Assessment of antifalcemic activity:

Inclusion criteria: To be included in this study, blood should be from homozygous sickle cell patients whose haemoglobin status has been proven by the haemoglobin electrophoresis method and who have not undergone blood transfusions within four months prior to the blood draw.

Collection and storage of blood samples: The collection of a 5 mL blood sample of whole blood on EDTA in a ratio of 1:5 (one volume of EDTA to four volumes of blood) is stored at 4 °C for no more than 8 days prior to use.

Emmel Review: The evaluation of the biological activity on sickle cell cells was carried out using the Emmel test summarized as follows: 5 mg of blood from the sickle cell is taken and mixed with 10 ml of physiological water (NaCl 0.9%) and then a few drops of this solution are tested with the extracts obtained under hypoxic conditions on slides. The control consists of diluted sickle cell blood without extract. The microscopic images were taken using a MOTIC light microscope after 30 minutes and a FUJI digital camera was used to digitize the micrographs.

RESULTS AND DISCUSSION

Phytochemical Screening: Phytochemical screening performed on aqueous and organic extracts of *C. cajan* revealed the presence of total polyphenols (tannins, quinones, flavonoids), alkaloids and sterols/polyterpenes. The results of phytochemical screening (tube or stained reactions) are described in Table 1.

Table 1: Chemical composition of dry seeds of *C. cajan*

Extracts	Chemical Groups							
	Sterols polyterpenes	Total polyphenols	Flavonoids	Tannins		Quinones	Alkaloids	
				Gal	Cat		D	B
Aqueous extract	-	+	+	+	+	-	-	-
Methanolic extract	+	+	+	+	+	+	-	-
Dichloromethane extract	+	±	±	-	-	+	+	+

- Gal = gallic; Cat = catechic; D = Dragendorff; B = Bouchardat
- (+) sign: indicates a positive reaction
- (-) sign: indicates a negative reaction
- (±) sign : indicates a trace presence

These results are similar to those of E. N'draman-Donou (2015) who conducted a study on the characterization of *C. cajan* seeds in Abidjan, Côte d'Ivoire. Chromatographic profiling on TLC revealed a diversity of chemical compounds, including polyphenols, flavonoids, tannins, sterols, and alkaloids in the extracts studied (Table 2).

Table 2: TLC Identification of Chemical Groups in Extracts

Extracts	Chemical Families	Colours of the spotlights		Rf
		In the visible	UV/366 nm	
Aqueous extract	Polyphenols	Red	Fluorescent Green	0.47
		Rose	Fluorescent Green	0.53
		Rose	Fluorescent Blue	0.56
		Brown	Orange	0.69
		Rose	Light Blue	0.75
	Flavonoids	Light Yellow	Yellow Green	0.23
		Pale yellow	Yellow Green	0.28
		Yellow	Red	0.47
		Yellow	Fluorescent Yellow	0.55
		Yellow		0.77
Methanolic extract	Anthracene compounds (quinones)	Yellow	Yellow-brown	0.15
		Violet	Purplish red	0.24
		Yellow	Greenish yellow	0.35
		Purple	Red	0.51

	Flavonoids	Yellow Yellow Pale yellow Ochre Yellow Yellow	Yellow Green Yellow Green Red Fluorescent Yellow Fluorescent Blue	0.27 0.31 0.45 0.54 0.57
Dichloromethane extract	Sterols polyterpenes	Mallow Rose Rose Mallow	Brown Orange Orange Orange-yellow	0.48 0.51 0.65 0.74
	Alkaloids	Yellow Yellow Yellow Red Purple Yellow	Fluorescent Blue Yellow Mallow Red-brown Orange Fluorescent Blue	0.29 0.38 0.42 0.54 0.66 0.74

The presence of flavonoids was confirmed by Neu's reagent, which made them appear in the visible light as yellow spots. Under UV/366 nm, these colours intensify and diversify. Compared to previous work (Wagner and Bladt, 1996, Mamyrbékova-Békro, 2008; Tekalé, 2016), it is inferred that the spots revealed may correspond to the flavonoids. The polyphenols were revealed by the Folin-Ciocalteu reagent. According to Wagner and Bladt (1996), yellow, green, and blue fluorescences can probably be flavanols or flavanones, respectively. Anthracene compounds are present in dichloromethane extract (purple, purple, yellow to visible coloration). Detection of these compounds by revealed thin-layer chromatography (with 5% methanolic KOH) brought out the red, yellow-brown, and greenish-yellow spots at different Rf. Sterols can be detected as yellow, purple, pink and orange-red spots depending on the visible or UV/366 nm detection. The detection of alkaloids by thin-layer chromatography revealed with the Dragendorff reagent revealed two very light spots in the visible range (Rf= 0.66, Rf=0.74) and three spots of less intense staining (Rf=0.38, Rf = 0.42, Rf=0.54). No in-depth phytochemical studies have been conducted on the species *C. cajan* from the Congo. However, in comparison with the work of Koffi Akessé Georges (2018), it should be noted that our results corroborate with the conclusions of this author.

Antifalcemic activity of extracts: Figures 3, 4, 5 and 6 below show micrographs of untreated SS blood and SS blood in the presence of aqueous, methanolic, and dichloromethane extracts.

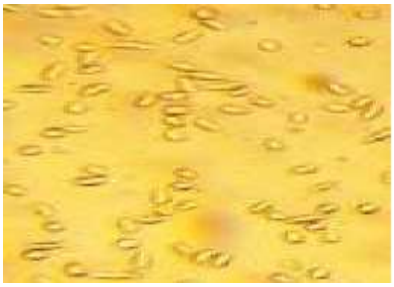

Fig.3: Sickle cell morphology of untreated SS blood

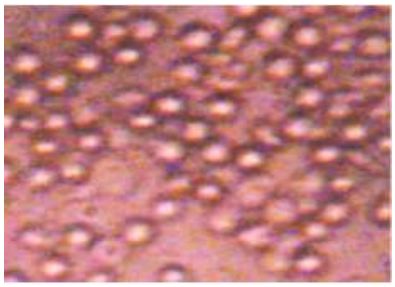

Fig. 4: Sickle cell morphology of SS blood treated with aqueous extracts

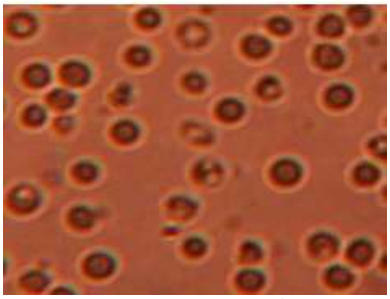

Fig. 5: morphology of the Sickle cells from SS blood treated with methanolic extracts

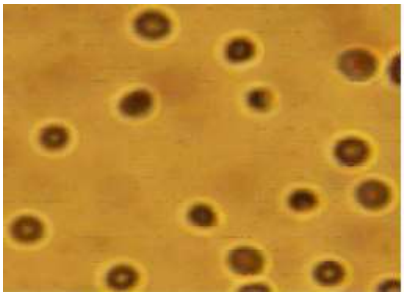

Fig. 6: morphology of the SS blood sickle cell treated with Dichloromethane extracts

The observation of the above figures shows that the different extracts of *C. cajan* have a remarkable activity on the sickling of red blood cells.Figure 3 shows that the majority of erythrocytes are sickle-shaped, but in the presence of the various extracts (Figures 3, 4 and 5) it appears that the erythrocytes take on the normal shape. This change in the shape of sickle cell cells was noticed in the presence of aqueous (large), methanolic (medium) and dichloromethane (low) extracts. This effect could depend on the nature of the extracts. Based on his observations and the work of Ekeke (1985), it can be noted that *C. cajan* inhibited the sickling of red blood cells in sickle cell patients. According to Ekeke (1990), *C. cajan* is able not only to inhibit the sickling of red blood cells but also to restore the normal shape of sickle cell red blood cells. Following this, a study demonstrated that a hydroalcoholic extract of *C. cajan* seeds did indeed have sickle cell properties but that the effect of this extract was concentration-dependent (Ogoda Onah, 2002). To be able to determine the chemical group responsible for this activity, we prepared the extracts in three solvents of increasing polarities, namely dichloromethane, methanol and water. Normalization of sickle cell cells (Figures 4, 5, 6) shows that anti-sickle cell activity increases with polarity. This indicates that the chemical group responsible for biological activity would be polar according to the "like dissolves like" principle. Given that polyphenols and flavonoids are polar substances and previous results (Mpiana, 2007a; 2007b; 2007c; Mpiana, 2008a; 2008b; Mpiana, 2009) have shown their antifalcemic activity, we can affirm that *C. cajan* is an antifalcemic plant. Polyphenols, flavonoids, and quinones have antioxidant properties that may be important in sickle cell anaemia, which is a disease that generates an excessive amount of free radicals. The antioxidant properties of a plant would therefore also indicate its action on sickle cell anaemia (Ngbolua, 2014). Indeed, flavonoids, like polyphenols in general, are known for their antioxidant activity. The presence of

these pigments is thought to contribute to the antioxidant properties of food plants used against sickle cell anaemia in traditional medicine. These properties would stabilize the erythrocyte membrane of SS blood and reduce the ratio of $Fe3^{+}/Fe^{2+}$ (Noguchi, 1978; Mpiana, 2011). Flavonoid compounds are thought to play the main role for the therapeutic effect on human health (Duker-Eshun, 2004; Mpiana, 2010). These results show that the extract can be used in the management of painful episodes experienced by sickle cell patients.

CONCLUSION AND APPLICATION OF RESULTS

It appears from this study that *C. cajan* is cited as one of the plants used against sickle cell anaemia. This plant has shown antifalcemic activity in vitro, thus confirming the information obtained in traditional medicine and in the literature. This plant could therefore be used as food to relieve those who suffer from this chronic disease. It is the seed that is mainly used for its anti-sickling action. The antifalcemic activity is thought to be due to the groups of chemicals contained in the plant. The main components responsible for this activity are phenolic acids. These components have an antifalcemia action but their mechanism of action is not known. It would therefore be interesting to study the mechanism of action of this plant now that some of the components responsible for the activity have been identified. In conclusion, *C. cajan* show promising results in the treatment of sickle cell anaemia.

Conflict of Interest

The authors state that there is no conflict of interest.

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