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Phytochemical profile and antioxidant, antibacterial, anti-inflammatory and anti-sickle cell activities of *Vigna radiata (L.) R.Wilczek* cultivated in DR Congo

[Profil phytochimique et activités antioxydante, antibactérienne, antiinflammatoire et antidrépanocytaire de *Vigna radiata (L.) R.Wilczek* cultivé en RD Congo]

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Abstract

Plants, especially medicinal varieties, play a vital role in human health. This study aims to analyze the phytochemical properties and evaluate the antisickling activity of Vigna radiata, cultivated in the Democratic Republic of Congo. Results indicate the presence of various chemical compounds, including total polyphenols (239.84 ± 0.57 mg EQ/g), flavonoids (8.089 ± 0.046 mg EQ/g), anthocyanins (0.503 ± 0.003 mg/g), hydrolyzable tannins (0.084 ± 0.002%), and condensed tannins (0.041 ± 0.001%), along with a foam index of 150 for saponins. The mineral composition, determined through X-ray fluorescence, revealed significant levels of Ca, K, Mg, Fe, Cu, Zn, and Mn. Extraction of V. radiata seeds was performed with solvents of increasing polarity, and the aqueous extract displayed notable antioxidant activity at 25 µg/mL, with an 81.92% inhibition rate. Furthermore, the DCM extract demonstrated anti-inflammatory effects at 3 mg/mL, inhibiting egg albumin denaturation by 22.72%. The aqueous extract exhibited antibacterial effects, inhibiting Escherichia coli (18 mm) and Salmonella SPS (22 mm), with Staphylococcus aureus showing intermediate sensitivity (10 mm), whereas Citrobacter diversus showed full resistance. Antisickling activity was significant in the aqueous extract, with normalization rates of 90%, 75%, and 60% at concentrations of 5 mg/mL, 2.5 mg/mL, and 1.25 mg/mL, respectively.

Keywords : *Vigna radiata*, anti-sickle cell, antioxidant, antibacterial, anti-inflammatory.

Résumé

Les plantes de manière générale, et médicinales en particulier jouent un rôle important dans la santé humaine. Ce travail a pour objectif l'étude phytochimique et évaluation de l'activité antidrépanocytaire de *Vigna radiata* cultivé en République Démocratique du Congo. Cette espèce a montré la présence des groupes chimiques tels que les polyphenols totaux (239,84 ± 0,57 mg EQ/g), flavonoïdes (8,089 ± 0,046 mg EQ/g), Anthocyanes (0,503 ± 0,003 mg/g), tanins hydrolysables et condensés (0,084 ± 0,002% et 0,041 ± 0,001%), Saponines (indice de mousse de 150), Alcaloïdes, Terpénoïdes, etc. le dosage des éléments minéraux par fluorescence X a révélé la présence de Ca, K, Mg, Fe, Cu, Zn, Mn, etc. L'extraction à polarité croissante a été effectuée sur la poudre des graines *V. radiata*. L'extrait aqueux a montré une forte activité antioxydante à 25µg/mL avec un taux d'inhibition de 81,923% ; l'extrait au DCM a montré une forte activité antiinflammatoire à 3 mg/mL avec un taux d'inhibition de dénaturation de l'albumine de l'œuf de 22,72% ; pour l'activité antibactérienne, deux souches se sont révélées sensibles à l'extrait aqueux, l'*Escherichia Coli* (18mm) par rapport à la ciprofloxacine et la *salmonelle SPS* (22 mm) par rapport à la levomicine, les germes de *staphylococcus aureus* ont présenté face à l'extrait aqueux une sensibilité intermédiaire (10mm) par rapport à la ciprofloxacine, Alors que les germes de *Citrobactere diversis* ont exhibé une totale résistance par rapport aux différents extraits ; l'activité antifalcémiante s'est révélée très bonne pour l'extrait aqueux avec 90%, 75% et 60% du taux de normalisation des globules rouges falciformes à des concentrations de 5 mg/mL, 2,5 mg/mL et 1,25 mg/mL respectivement.

Mots clés : *Vigna radiata*, antidrépanocytaire, antioxydant, antibactérien, antiinflammatoire.

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1. Introduction

The natural environment is crucial for both humans and animals, providing oxygen, nutrients, and favorable climates. Since ancient times, medicinal plants have been invaluable for treating various diseases, such as tuberculosis, sickle cell anemia, diabetes, hypertension, and cancer. This effectiveness is due to a wide range of secondary metabolites like polyphenols, saponins, alkaloids, steroids, terpenoids, and essential primary metabolites, including mineral salts, vitamins, proteins, and lipids (Kitadi et al., 2019a; Matondo et al., 2021)

In the pursuit of new treatments, scientists focus on the therapeutic properties of these plants to isolate bioactive compounds for developing targeted medications.

In a context where chronic and infectious diseases, such as bacterial infections, inflammations and sickle cell disease, constitute a major public health challenge in the DRC, the search for accessible and effective treatments has become essential. Furthermore, the synthetic derivatives used to treat these conditions sometimes show limitations, such as microbial resistance and side effects. In this perspective, medicinal plants offer an interesting alternative, because they contain bioactive secondary metabolites likely to act on several biological targets. (Ngbolua et al., 2020; Mpiana et al., 2020)

Belonging to the Fabaceae family, the genus Vigna is widely cultivated in tropical regions, particularly in Southeast Asia, and Western and Central Africa (An et al., 2020; Kumar and Singhal, 2009). With over 200 species, Vigna radiata (commonly known as "kambululu" in the Tshiluba language of the DRC) is widely consumed and valued for its nutritional and medicinal benefits. Rich in primary metabolites (such as vitamins and proteins) and secondary metabolites (including polyphenols and saponins), V. radiata has shown potential for managing various conditions, including sickle cell anemia, which affects over five million people, primarily in sub-Saharan Africa (Priya et al., 2012; Saini et al., 2010 ; Mpiana et al., 2010).

Sickle cell patients develop many health problems such as hemolysis which causes vascular crises, pain crises due to obstruction of blood vessels, risk of infections, etc. Because of all these problems, the introduction of antioxidant, anti-inflammatory and antibacterial agents in the management of sickle cell patients is of paramount importance.

This study aims to evaluate the phytochemical profile and biological activities, including antioxidant, antibacterial, anti-inflammatory and antisickling properties of V. radiata cultivated in the DRC. Such an approach not only allows to valorize this endemic plant but also to contribute to the search for therapeutic solutions based on local resources.

2. Materials et methods

2.1. Material

2.1.1. Plant material

The plant material consists of the seeds of *Vigna radiata* (Kambululu in Tshiluba). These seeds were purchased near the University of Kinshasa and were then identified at the Herbarium of the National Institute of Agronomic Studies and Research (INERA).

2.1.2. Blood

The sickle cell blood used was collected at the Mixed Medicine and SS Anemia Center commonly called the Mabanga Center located in the city of Kinshasa (DRC) with the patients' permission.

2.1.3. Microbial strains

The microbial strains used in this work were cultivated in the microbiological laboratory of the Ngaliema clinic in the city of Kinshasa (DRC). They consist of strains of: *Escherichia Coli*, *Salmonella SPS*, *Staphylococus aureus* and *Citrobactere Diversis*.

2.2. Methods

2.2.1. Phytochemical study

- Chemical screening

V. radiata seeds were macerated in distilled water (proportion 1:10 for 24 h) and in methanol (proportion 2:10 for 24 h) to identify the secondary metabolites present following the standard protocol.

Thin layer chromatography (TLC) using silica gel was performed according to the protocol outlined by Wagner (Wagner et al., 1984; Ngoyi et al., 2020; Kabengele et al., 2020; Bruneton 1999) based on the observation of spots of various colors to identify different secondary metabolites.

- Total polyphenol dosages

The determination of the total polyphenol content of the methanolic extract is carried out using the spectrophotometric method of dosage by the Folin-Ciocalteu test (Kabengele, et al., 2022)

- Dosage of flavonoids

The total flavonoid content was quantified using the method described by Lebreton (Lebreton et al., 1967; Kasiama et al., 2022).

- Anthocyanin dosage

The dosage of anthocyanins was carried out according to the Lebreton methodology (Lebreton et al., 1967; Kasiama et al., 2022).

- Dosage of hydrolyzable tannins

The content of hydrolyzable tannins was determined by the Mole method based on a reaction with ferric chloride.

- Dosage of condensed tannins

This dosage is based on the condensation of polyphenolic compounds with vanillin in an acid medium (Dohou et al., 2003).

- Saponin dosage

The presence of saponins is quantitatively determined by calculating the foam index.

- Dosage of mineral elements by X-ray fluorescence

The plant powder was homogenized by vigorous shaking in the plastic bag. A test portion was prepared by mixing 4.2155 g of sample powder with 0.8 g of Fluxana, and Cereox from Höchstwax as a binder (i.e., 20% of the sample mass), before compressing it in a "Carver" pelletizer. IPE-10-31 and BCR-62 pellets were used as reference materials to determine errors.

The pellets were then positioned in the sample changer plate, and X-ray fluorescence spectra were obtained using secondary targets. The functionality depended on parameters such as working voltage, excitation energy, etc. (Ngoyi et al., 2020; Kitadi et al., 2019b)

- Extraction with increasing polarity

100 g of *V. radiata* powder were sequentially extracted, beginning with 250 mL of petroleum ether. After 24 hours of maceration and filtration, the ether fraction (E-EP) was obtained. The residue was then treated with dichloromethane to obtain the dichloromethane fraction (E-DCM), followed by methanol (E-methanol), and finally distilled water (aqueous extract). Each step was repeated twice. The fractions were evaporated using a rotary evaporator until the solvent was completely removed, then dried in an oven at 40°C (Gbolo et al., 2023).

2.2.2. Biological activities

This study aimed to evaluate the antioxidant activity using the DPPH° assay (Bongo et al., 2017; Tshilanda et al., 2019), in vitro anti-inflammatory activity (Samira et al., 2020), antibacterial activity (Ikey et al., 2022) and anti-sickling activity using the Emmel test (Kambale et al., 2013).

3. Résultats et Discussion

3.1. Phytochemical study

3.1.1. Chemical screening

Table 1 below shows the results of the chemical screening of V. radiata cultivated in the DRC.

Table 1. Result of chemical screening

Phytochemical groups		*Vigna radiata*
Polyphenols		+
Flavonoids		+
Related Quinones		+
Anthocyanins		+
Leuco-anthocyanin		+
Tannins		+
Alkaloids		+
Saponins		+
Free Quinones		+
Terpenoids	Triterpenoids	+
	Steroids	+

Legend: (+) Positive test and (-) Negative test.

Solution screening revealed the presence of several secondary metabolites in the sample. Indeed, medicinal plants contain these compounds, which contribute to their therapeutic properties (Mpiana et al. 2009 a, b).

The TLC analysis of V. radiata seeds produced the results shown in Figure 1 below.

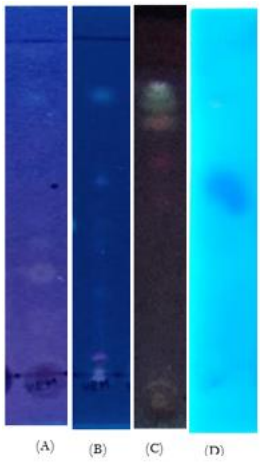


Figure 1. A, B, C, and D. Chromatogram of the organic extract for flavonoids, triterpenoids, alkaloids, and coumarins, respectively.

***Mobile phase**: Ethyl acetate-formic acid-glacial acetic acid-water (50:6.5:6.5:13.5); Toluene/ethyl acetate (9:1); Toluene-ethyl acetate-diethylamine (35:10:5) for flavonoids, triterpenoids, and alkaloids, respectively.*

***Revelation**: After development, the chromatoplate is observed under UV at 366 nm, then the Neu reagent is sprayed, followed by observation under UV at 366 nm. The presence of flavonoids is visualized by blue fluorescent spots.*

***Revelation (for other compounds)**: Anisaldehyde sulfuric reagent is used, followed by heating at 100°C for 10 minutes.*

TLC analysis confirmed the presence of flavonoids, triterpenes, alkaloids, and coumarins in *V. radiata* seeds. In both species, polyphenolic compounds were identified, including flavonoids (Rf = 0.1 and 0.75), coumarins (Rf = 0.5), triterpenes (Rf = 0.3), and alkaloids (Rf = 0.75, 0.83, 0.9, and 0.95). This supports their potential classification as functional foods for managing sickle cell disease. (Mpiana et al., 2016).

3.1.2. Dosage of secondary metabolites

Table 2 below presents the results of the quantification of secondary metabolites.

Table 2. Total metabolities in the V. radiata seeds

Secondary metabolites	Concentration (content)
Total polyphenols	239.84 ± 0.57 mg EQ/g
Total flavonoids	8.089 ± 0.046 mg EQ/g
Anthocyanins	0.503 ± 0.003 mg/g
Hydrolyzable and condensed tannins	0.084 ± 0.002% and 0.041 ± 0.001%
Saponins	Foam index of 150

It is clear from this table 2 that the species of V. radiata cultivated in the DRC presents good quantities of secondary metabolites, which would give this plant its numerous therapeutic virtues. The presence of these compounds in good proportion in this species of Vigna has also been observed in certain countries such as Burkina-Faso (Ouedraogo et al., 2021), in India (Shrinivas et al., 2020).

3.1.3. Dosage of mineral elements by X-ray fluorescence

Figure 2 shows the mineral content of *V. radiata*.

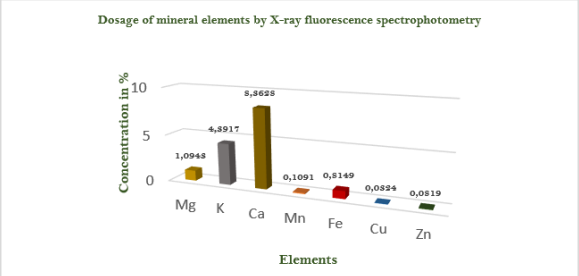


Figure 2. Mineral element content of V. radiata

Figure 2 clearly indicates that Vigna radiata seeds are rich in essential mineral elements, including magnesium (Mg), iron (Fe), potassium (K), calcium (Ca), and zinc (Zn), among others. Notably, calcium is present at a relatively high concentration (8.8628%), while other elements, such as zinc (Zn) and copper (Cu), are found in trace amounts. The mineral composition of Vigna radiata seeds has been similarly observed in other regions, though the specific proportions vary, likely influenced by local soil composition and climatic conditions. (Nwar et al., 2007).

Examining the mineral composition of Vigna radiata is crucial, as it provides valuable insights into the plant's nutritional benefits. Mineral elements are essential for various physiological functions. For instance, iron (Fe), abundant in V. radiata seeds, is vital for the structure of red blood cells and helps prevent anemia. Zinc (Zn) and copper (Cu) serve as antioxidants and are key to strengthening the immune system (Sguera, 2008). Calcium (Ca) is essential for bone and tooth composition, supporting their strength, while Ca and magnesium (Mg) also contribute to the proper functioning of the nervous system and muscle contractions. Additionally, Mg plays a significant role in managing diabetes and high blood pressure, enhancing overall health. (Ngoyi et al., 2020; He et al., 2006).

3.1.4. Extraction yield

Figure 3 presents the extraction yield obtained from 100 grams of *Vigna radiata* powder when subjected to a sequence of solvents with increasing polarity.

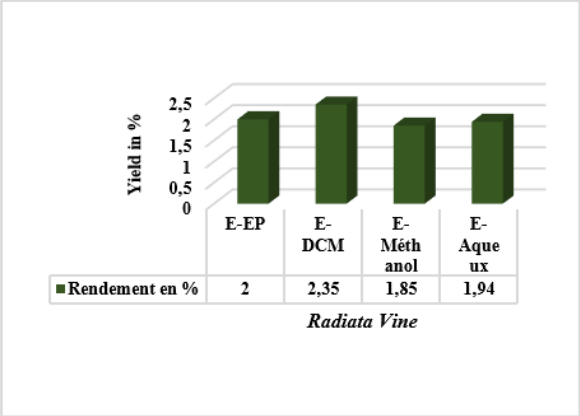


Figure 3. Extraction yield in %

According to Figure 3, the dichloromethane extract (E-DCM) achieved the highest yield at 2.35%, followed closely by the petroleum ether extract (E-EP) at 2.00%, the aqueous extract (E-Aqueous) at 1.94%, and finally the methanol extract (E-Methanol) at 1.85%. These extraction yields suggest that *Vigna radiata* seeds are richer in apolar compounds, as shown by the higher yields with non-polar solvents like dichloromethane and petroleum ether. The lower yields from polar solvents such as water and methanol

indicate a relatively smaller proportion of polar compounds in the seeds.

3.2. Biological activities

3.2.1. Antioxidant activity

Figure 4 illustrates the DPPH° radical inhibition percentages for various *Vigna radiata* extracts at a concentration of 25 µg/mL.

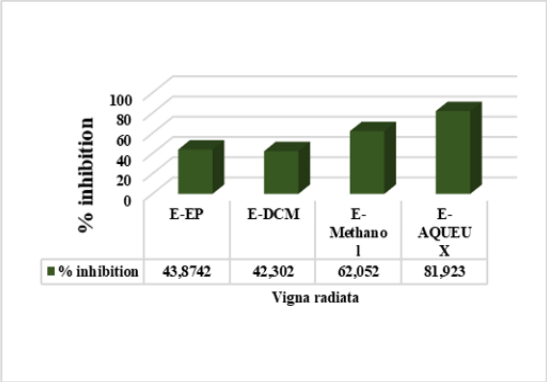


Figure 4. Percentage of inhibition of the DPPH° radical of different extracts of V. radiata

Figure 4 reveals that the aqueous extract of *Vigna radiata* exhibits a very high DPPH° radical inhibition percentage of 81.923% at a concentration of 25 µg/mL. This strong antioxidant activity can be attributed to the polyphenolic compounds present in the species, which are known for their ability to neutralize free radicals. Given that *V. radiata* is an edible species, consuming it could offer the population significant antioxidant benefits. Additionally, it is observed that the percentage of inhibition tends to increase with the polarity of the solvents used. This trend aligns with findings from similar studies on *V. radiata* cultivated in India (Shrinivas et al., 2020).

3.2.2. Anti-inflammatory activity

Figure 5 displays the percentage inhibition of egg albumin denaturation by different *Vigna radiata* extracts at a concentration of 3 mg/mL.

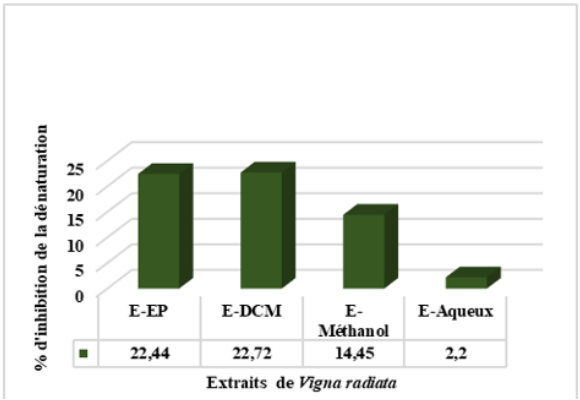
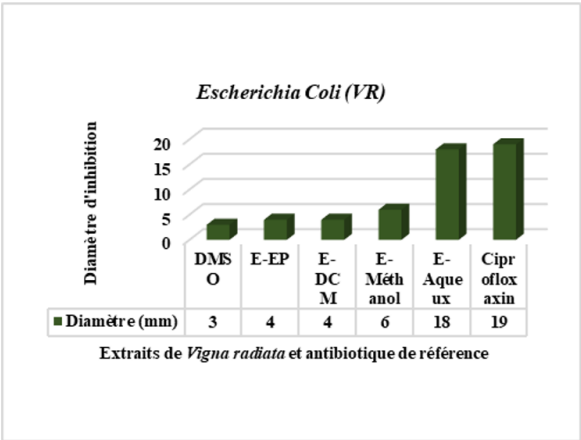


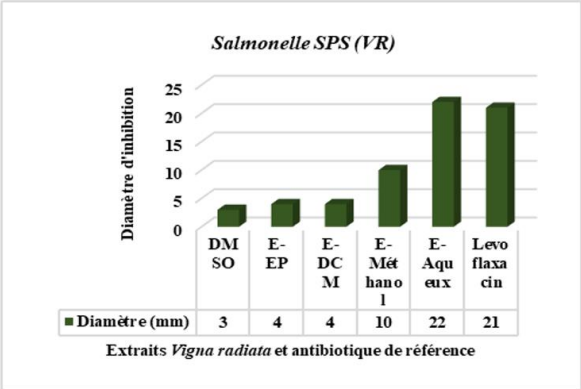
Figure 5. Percentage inhibition of egg albumin denaturation by V. radiata extracts

Figure 5 demonstrates that the dichloromethane extract exhibits the highest percentage of inhibition in preventing egg albumin denaturation, followed by the petroleum ether extract, then methanol, and finally the aqueous extract. These results suggest that the anti-inflammatory properties of *Vigna radiata* seeds are primarily attributed to its apolar bioactive compounds, such as steroids and triterpenoids. These compounds, which are more effectively extracted by non-polar solvents like dichloromethane and petroleum ether, likely play a key role in the plant's ability to mitigate inflammation (De Cassia et al., 2013)

3.2.3. Antibacterial activity

Figure 6 presents the inhibition diameters of various *Vigna radiata* extracts against different bacterial strains, including *Escherichia coli*, *Salmonella spp*, *Staphylococcus aureus*, and *Citrobacter diversus*.





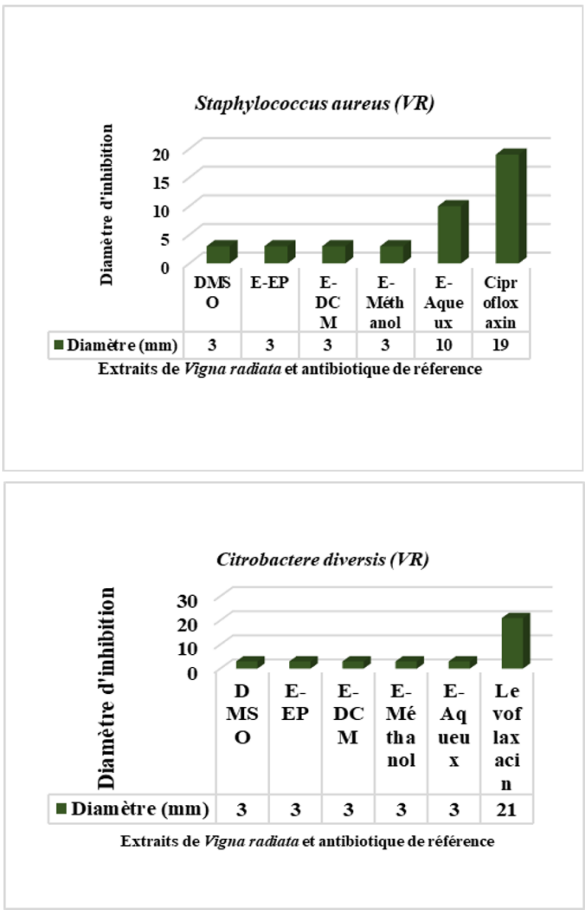


Figure 6. Inhibition diameter of different V. radiata extracts

The antibacterial activity of Vigna radiata seed extracts, as shown by the inhibition diameters, reveals some interesting findings. The aqueous extract was effective against *Escherichia coli* (18 mm) and *Salmonella spp.* (22 mm), with inhibition comparable to that of ciprofloxacin and levomicin, respectively. *Staphylococcus aureus* showed a moderate sensitivity to the aqueous extract, with an inhibition diameter of 10 mm, while *Citrobacter diversus* exhibited complete resistance to all extracts tested.

These results suggest that the antibacterial properties of V. radiata seeds are primarily attributed to its polar compound. The effectiveness of the aqueous extract against certain bacterial strains indicates that the binding sites on the bacterial receptors are likely hydrophilic in nature, interacting with the polar chemical groups in the compounds present in the seed extracts. This highlights the potential of V. radiata as a source of natural antibacterial agents, particularly against Gram-negative bacteria like *E. coli* and *Salmonella.*

3.2.4. Antisickling activity

Figure 7 presents micrographs comparing untreated sickle cell (SS) blood with blood treated using different *V. radiata* extracts.

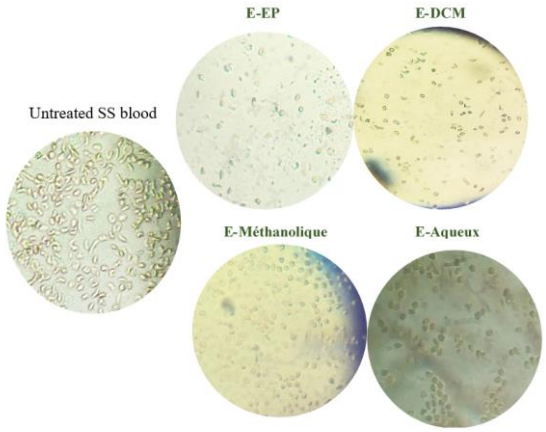


Figure 7. Micrographs of untreated SS blood and blood treated with the different V. radiata extracts

In the control sample, as seen in Figure 7, nearly all the erythrocytes exhibit the sickle shape, characteristic of sickle cell disease. However, after treatment with *Vigna radiata* extracts, a significant number of red blood cells have returned to their normal biconcave shape. This change suggests that *V. radiata* extracts may have a beneficial effect in alleviating the sickling of red blood cells, potentially improving the condition of individuals with sickle cell disease.

Figure 8 shows the normalization rate, which quantifies the extent of this shape restoration

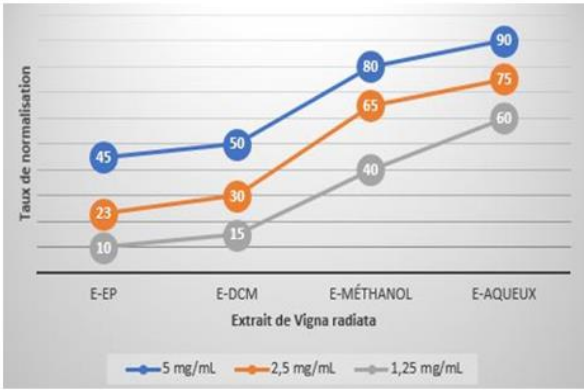


Figure 8. Normalization rate in the presence of V. radiata extracts

The antifalciform activity of *Vigna radiata* extracts, demonstrated through the Emmel test, reveals that the normalization of sickle-shaped red blood cells improves with the polarity of the solvents used. This finding suggests that the polar bioactive compounds, particularly polyphenolic compounds, are primarily

responsible for the antifalciform effects of *V. radiata* seeds, as supported by previous studies (Mpiana et al., 2012; Mpiana et al., 2016; Tshilanda et al., 2015; Ngbolua et al., 2014).

Additionally, the normalization rate increases positively with the concentration of each extract, indicating that higher concentrations enhance the effectiveness of *V. radiata* extracts in reversing sickling, further suggesting their therapeutic potential for sickle cell disease.

4. Conclusion

This study is part of a broader research program focused on Congolese medicinal plants, particularly within the genus *Vigna*, with a specific emphasis on *Vigna radiata*. The phytochemical analysis of *V. radiata* revealed a rich composition of bioactive compounds, including polyphenols, flavonoids, anthocyanins, saponins, and triterpenoids. Quantitative analyses showed significant levels of these compounds: 239.84 ± 0.57 mg EQ/g of total polyphenols, 8.089 ± 0.046 mg EQ/g of total flavonoids, 0.503 ± 0.003 mg/g of anthocyanins, 0.084 ± 0.002% hydrolyzable tannins, and 0.041 ± 0.001% condensed tannins, with a foam index of 150 for saponins. Mineralogical analysis identified seven key elements in the seed powder: calcium (Ca), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu).

The antioxidant activity of *V. radiata* evolved with the polarity of the solvents used, with the aqueous extract exhibiting the highest activity, achieving a normalization rate of 81.923%. This antioxidant effect is largely attributed to the polyphenolic compounds in the plant. The anti-inflammatory activity, as indicated by the inhibition of egg albumin denaturation, is primarily due to the apolar bioactive compounds, with the petroleum ether and dichloromethane extracts showing the strongest effects.

In terms of antibacterial activity, *V. radiata* seeds demonstrated significant antimicrobial properties, particularly against *Escherichia coli* and *Salmonella spp*, which showed high sensitivity to the aqueous extract. *Staphylococcus aureus* exhibited intermediate sensitivity, while *Citrobacter diversus* was completely resistant to all extracts. The antimicrobial activity suggests that the polar bioactive compounds, such as polyphenols, play a key role in combating these pathogens.

Finally, the antifalciform activity of *V. radiata* seeds was shown to correlate with the polarity of the extraction solvents, with the normalization of sickle-shaped red blood cells improving as the polarity increased.

These findings underscore the strong therapeutic potential of *V. radiata* seeds. In addition to their well-known nutritional benefits, they have demonstrated promising therapeutic properties, particularly in the context of sickle cell disease, where they could serve as a valuable palliative treatment. Given these multiple health benefits, *V. radiata* seeds warrant further valorization and exploration as a natural therapeutic agent.

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