

Full Length Research Paper

Qualitative, quantitative composition and evaluation of the antioxidant activity of polar extracts of stem bark of *Hexalobus crispiflorus* A. Rich (Annonaceae family): Central African species

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Hexalobus crispiflorus A. Rich., synonym *Hexalobus grandiflorus* Benth, is a plant in the Central African pharmacopoeia, widely used in the treatment of skin diseases and diabetes. The present study is a contribution to the qualitative and quantitative analysis and evaluation of the antioxidant activity of this plant. Preliminary phytochemical tests carried out on the different crude extracts of *H. crispiflorus* revealed the presence of flavonoids, anthocyanins, tannins, saponosides, triterpenes and sterols, followed by confirmation by thin layer chromatography. Quantitative analysis showed that the hydroethanolic extracts (50%) of *H. crispiflorus* have a high content of total polyphenols and flavonoids $955.11 \pm 0.76 \mu\text{g AG/g Ms}$ and $779.85 \pm 7.93 \mu\text{g Eq Quer/g Ms}$, respectively. Hydroethanolic extract remains the best solvent for extraction of phenolic compounds. The evaluation of the free radical scavenging capacity using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical trapping method on 96-well microplates showed that only the hydroethanolic (50%, 80%) and aqueous extracts had a moderate percentage of inhibition 17.94, 19.47 and 39.85%, respectively, but this was still low compared to the reference molecule, ascorbic acid (89.4%). The results obtained could justify the therapeutic virtues of this species in the treatment of various pathologies.

Key words: *Hexalobus crispiflorus* A. Rich, extracts, qualitative and quantitative composition, antioxidant activity.

INTRODUCTION

Today, reliance on medicinal plants is particularly pronounced in developing countries where modern

medicine is unavailable or too expensive. Despite advances in biology and modern medicine, the vast

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Figure 1. Location of *H. crispiflorus* harvesting area.
Source: LACCEG (2015).

majority of people in developing countries do not have access to adequate healthcare due to their low economic income (Kouchadé et al., 2017; WHO, 2012). In sub-Saharan countries in general, and in the Central African Republic in particular, a large proportion of the population turns to medicinal plants for treatment because their poverty makes it difficult for them to access the benefits of modern medicine.

In the Central African Republic, as in many other African countries, nearly 80% of the rural population relies on traditional herbal remedies to treat themselves (Tijjani et al., 2017; Kouchadé et al., 2017), as this medicine is the most economically, geographically and culturally accessible.

The Central African flora is rich in medicinal plants, traditionally used for their many therapeutic virtues. Today, in Africa and particularly in the Central African Republic, studies on several plants have demonstrated their therapeutic virtues, including those of *Hexalobus crispiflorus* A. Rich.

H. crispiflorus A. Rich belongs to the Annonaceae family, which comprises more than 2,550 species and 113 genera (Couvreur et al., 2022) and is native to tropical Africa. It is a medium-sized tree that can reach 30 to 35 m in height and 1.7 m in girth. It is found in countries such as Cameroon, Equatorial Guinea, Gabon, DR Congo, Ivory Coast and Senegal (Okhale et al., 2016). It has been reported that *H. crispiflorus* A. Rich, contains saponosides, sterols, and triterpenes. The vegetative organs of *H. crispiflorus* A. Rich, are used to treat skin diseases, eye diseases, fever and wounds, diabetes, and candidiasis (Hamisi et al., 2014).

In the Central African Republic, a great deal of work has been devoted to ethnobotanical inventories in order to contribute to the knowledge of this plant. In order to

make the most of *H. crispiflorus*, it is necessary to carry out phytochemical, pharmacological, and chemical research. With this in mind, we set ourselves the objective of carrying out a qualitative and quantitative analysis and evaluation of the antioxidant activity of polar extracts of the bark of *H. crispiflorus* A. Rich.

MATERIALS AND METHODS

Plant

Bark from the stem of *H. crispiflorus* A. Rich, was used. It was collected in June 2022 in the village of Bagandou in the Lobaye region (Figure 1), south of Bangui (Central African Republic). The plant was botanically identified at the Plant Biodiversity Laboratory of the Faculty of Science at the University of Bangui. The bark of the trunk was dried in a dark, dry place and then ground into a powder in a traditional mortar. The powder obtained was stored in glass bottles for later analysis.

Preparation of extracts

Two grams of *H. crispiflorus* A. Rich, were cold macerated in 40 mL of ethanol, ethanol-water (80%, v/v), ethanol-water (50%, v/v) and aqueous under magnetic stirring for 24 h. After filtration, the different filtrates obtained were stored in a cool place at -4°C for the different analyses.

Preliminary phytochemical analysis

The main families of secondary metabolites were identified by tube colour reactions using the classical methods described by Ngaïssona et al. (2016), Mahadeva et al. (2016), and Onuminya et al. (2017) and by thin layer chromatography. Tannins and polyphenols were identified by the ferric trichloride (FeCl₃) test and Stiasny's reagent, flavonoids by the cyanidine reaction, saponosides by the foam test, triterpenes and sterols by the Liberman-Bürchard

test, alkaloids by the Draggendorf and Mayer tests and finally reducing sugars by Fehling's liqueur test.

The qualitative composition of plant extracts can be determined by thin layer chromatography (TLC). Analyses are carried out in the normal phase on silica plates (Silica gel 60 F254, thickness 0.25 mm) in a thin layer on an aluminium plate, which constitutes the stationary phase. Five microliters each of crude extract (1 g in 15 ml of solvent, giving a concentration of 66.67 mg/ml) were deposited, the deposits were dried with a hairdryer, the plates examined under a UV lamp at 254 and 365 nm and then developed with a specific developer.

Binary and non-binary solvent systems were used depending on the chemical groups of interest:

- (1) Ethyl acetate/Methanol/Ammonia (9/1/1, v/v/v) for alkaloids, Draggendorf reagent developer;
- (2) Petroleum ether/Ethyl acetate (7/3, v/v) for triterpenes and sterols, anisaldehyde or Liberman-Bürchard developer;
- (3) Ethyl acetate/Formic acid/Water (8/1/1, v/v/v) for flavonoids, 1% AlCl_3 developer;
- (4) Ethyl acetate/Formic acid/Water (8/1/1, v/v/v) for tannins, coumarins, Fast Blue Salt B developer.

Quantitative analysis

Determination of total polyphenols

The total polyphenol content was determined spectrophotometrically, using the colorimetric method with the Folin-Ciocalteu reagent (Mahadeva et al., 2016; Kouamé et al., 2021) with a slight modification. This assay is based on the quantification of hydroxyl groups present in the different extracts (ethanol 100%; ethanol-water 80%, v/v; ethanol-water 50%, v/v) and aqueous extracts.

In test tubes, a volume of 100 μL each of ethanolic, hydroethanolic and aqueous extracts from the bark of the trunk of *H. crispiflorus* was diluted with 900 μL distilled water, then with 900 μL Folin-Ciocalteu (1 N), and 200 μL sodium bicarbonate solution (20%) was added to the mixture. The tubes were vortexed and kept in the dark for 40 min. Absorbance was measured at 725 nm using a JENWAY 6300 spectrophotometer against a methanol solution used as a blank.

A calibration curve ($y = ax + b$) was established under the operating conditions using gallic acid at different concentrations (0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml). The results obtained were expressed in equivalent micrograms of gallic acid per gram of dry matter ($\mu\text{g Eq AG/g MS}$). All assays were repeated at least three times for each sample.

Determination of total flavonoids

The flavonoid content of the crude extracts was determined by the aluminium trichloride method. The protocol used is based on that described by Rachedi et al. (2018), Dirar et al. (2019), and Kouamé et al. (2021) with some modifications.

Two hundred and fifty microliters of each extract and 1000 μL are added successively to test tubes. At time zero ($t = 0$), 75 μL of a 5% sodium nitrite (NaNO_2) solution was added to the mixture. After 5 min ($t = 5$ min), 75 μL of a 10% aluminium trichloride solution was added. The mixture was stirred and allowed to stand at room temperature for 6 min. Then 500 μL NaOH (1 N) and 2.5 mL distilled water were added to the reaction mixture, which was then vortexed vigorously. The absorbance was read at 510 nm using a JENWAY model 6300 spectrophotometer against a methanol solution used as a control.

A calibration curve ($y = ax + b$) established with different

concentrations of quercetin (0.02, 0.04, 0.06, 0.08 and 0.1 mg/ml), carried out under the same working conditions as the extracts, is used to quantify the flavonoids. The results obtained were expressed as micrograms of quercetin equivalent per gram of dry matter ($\mu\text{g Eq Quer/g MS}$). All assays were repeated at least three times for each sample.

Determination of condensed tannins

The condensed tannin content was determined using the vanillin method in acid medium (Rachedi et al., 2018; Kouamé et al., 2021).

A volume of 50 μL of each crude extract was added to 1500 μL of vanillin/methanol solution (4%, w/v) and then vortexed. Next, 750 μL of concentrated hydrochloric acid solution was added to the mixture and allowed to react for 20 min at room temperature. The absorbance is read at 550 nm against a methanol solution used as a blank. The concentration of tannins is estimated in equivalent micrograms of catechin per gram of dry matter from the calibration curve ($\mu\text{g Eq Cat/g Ms}$).

Assessing antioxidant activity

A compound with antioxidant activity turns yellow in the presence of the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (violet) by donating a proton to the radical.

DPPH method in a 96-well microplate

This method is based on the use of a free radical: DPPH, as described by Dieng et al. (2017) and Rahul et al. (2022) with a slight modification. The reduction of DPPH is accompanied by a change in the colour of the solution from purple to yellow, which can be measured spectrophotometrically at 525 nm. The intensity of the colour change measured by the spectrophotometer is inversely proportional to the antioxidant activity of the extracts whose activity is to be determined.

For our extracts, we prepared a solution of DPPH at a concentration of 7 mg/20 ml in methanol. This solution was diluted 10 times and stored in the refrigerator for a maximum of two days. From the stock solution of each extract (EtOH, EtOH-water (80%), EtOH-water (50%), and aqueous), four dilutions were prepared at different concentrations (60, 150, 240 and 300 mg/L). A pasteurised pipette was used to add 20 μL of each extract and 180 μL of DPPH to each microplate well. The blank was 20 μL DMSO and 180 μL MeOH. Readings were taken at 524 nm after 25 min incubation in the dark. Ascorbic acid was used as a standard, prepared under the same conditions as the extracts. All extracts were reproduced at least 4 times to minimise error. The results were expressed as percentage inhibition (%). Antioxidant activity can be characterised by a variable called IC_{50} (50% inhibitory concentration), which is the concentration of test sample required to reduce 50% of the DPPH radical.

The IC_{50} values were calculated graphically using the linear regression method for the graphs tested, showing the percentage of inhibition as a function of the different concentrations of crude extracts.

RESULTS AND DISCUSSION

Qualitative composition by tube reaction

The identification of the different classes of secondary metabolites present in the bark of the trunk of *H.*

Table 1. The chemical profiles of extracts from the bark of the stem of *Hexalobus crispiflorus* A. Rich.

Chemical groups	<i>H. crispiflorus</i> A. rich bark
Alkaloids	-
Flavonoids	+++
Anthocyanins	+++
Tannins	+++
Catechin tannins	+++
Gallic tannins	-
Quinones	+++
Anthracene derivatives	+++
Saponins	+++
Foam index	1500
Triterpenes and stérols	+++
Reducing sugars	+++

+++ : Presence; - : Négative.

crispiflorus has given us a good idea of its pharmacological activities.

The results of the preliminary tests carried out on the crude extracts of the trunk of *H. crispiflorus* A. Rich., are shown in Table 1. It was noted that flavonoids, anthocyanins, anthracene derivatives, quinones, catechic tannins, saponins, triterpenes and sterols, as well as reducing sugars, are very abundant in the crude extracts of this plant. Alkaloids and bile tannins are completely absent.

The results of phytochemical screening show that the stem barks of *H. crispiflorus* A. Rich., are rich in secondary metabolites as shown in a previous phytochemical study (Ehiabhi Okhale et al., 2016; Abderaman et al., 2018; Akotegnon et al., 2018). The presence of these chemical families in the leaves and roots of varieties of the species from Benin and Nigeria has also been demonstrated (Akotegnon et al., 2018; Abderaman et al., 2018). However, the absence of alkaloids and gall tannins in the Central African Republic variety was noticed.

Qualitative composition by thin layer chromatography (TLC)

Identification of flavonoids

After revelation with aluminium trichloride (1%) and observation under UV light at 366 nm, Figure 2 below shows staining: yellow, greenish yellow and bluish white in the polar extracts of *H. crispiflorus* are due to flavonoids, the light green, blue and bluish white bands are due to phenolic acids (probably coumarins).

Identification of terpenoids

Figure 3 shows the of the apolar extracts prepared

according to Libermann-Bürchard and then heated to 105°C show the presence of grey, violet-blue and brown spots in the trunk bark of *H. crispiflorus*, due to triterpenes, and the green and yellow-green bands due to sterols.

Identification of tannins and coumarins

Figure 4 below shows chromatograms of polar extracts of the stem bark of *H. crispiflorus* confirm the presence of tannins in this species, with the appearance of grey and brown spots after spraying with Fast Blue Salt B reagent. The yellow and green stains are due to coumarins.

Alkaloids research

Figure 5 below shows the presence of blue fluorescent spots in the polar extracts of the bark of the stem of *H. crispiflorus* under a UV lamp at 366 nm, as well as the yellow-orange spots observed in visible light after spraying with Dragendorff's reagent. The yellow-orange spots indicate the presence of alkaloids in the organ of this species.

The results of the thin layer chromatography confirm the presence of certain chemical families observed in the tube reactions, in particular flavonoids, tannins, terpenes and sterols. Previous work on the different organs of *H. crispiflorus* A. Rich., had shown the presence of alkaloids, triterpenes, and flavonoids in species of the Nigerian variety (Ehiabhi et al., 2016).

Total polyphenol, flavonoid and condensed tannin content

The determination of total polyphenols, flavonoids and

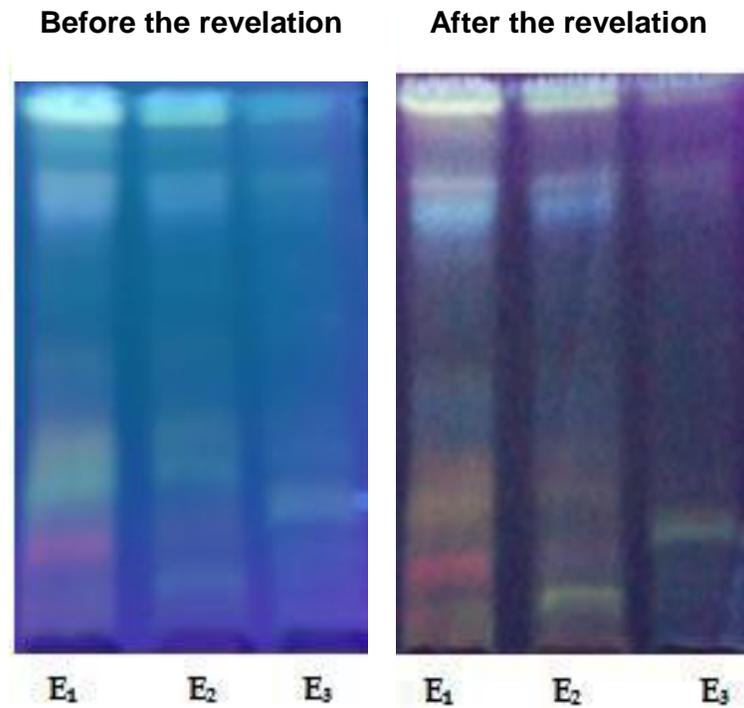


Figure 2. First chromatogram of TLC results for polar extracts from the stem barks of *H. crispiflorus* A. Rich. Developer: Aluminium trichloride (1%) E₁: EtOH; E₂: EtOH-H₂O; E₃: aqueous.

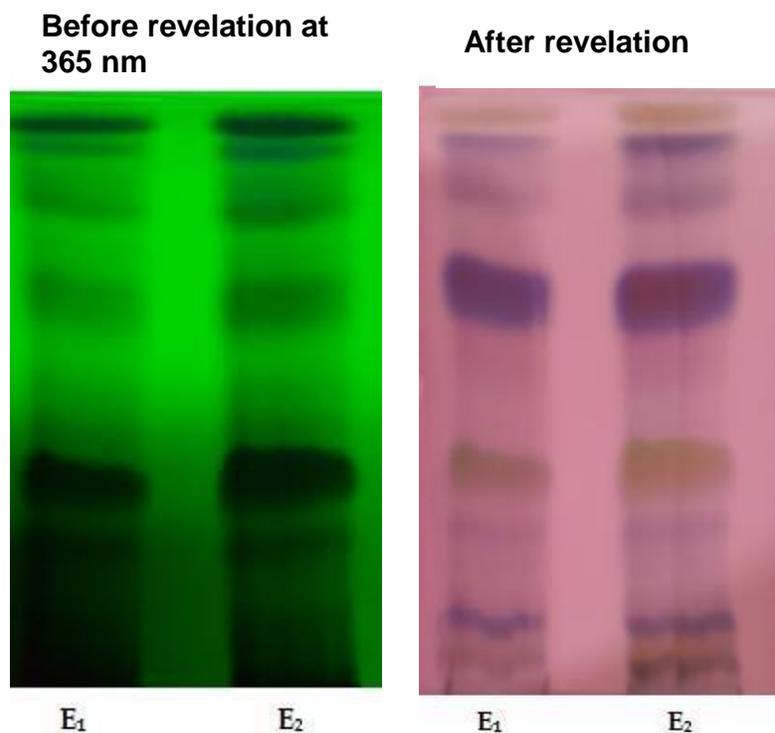


Figure 3. Second chromatogram of TLC result for terpenoids in apolar extracts the stem bark of *H. crispiflorus* A.Rich. Developer: Liberman then heating to 105°C; E₁: chloroform extract; E₂: dichloromethane extract.

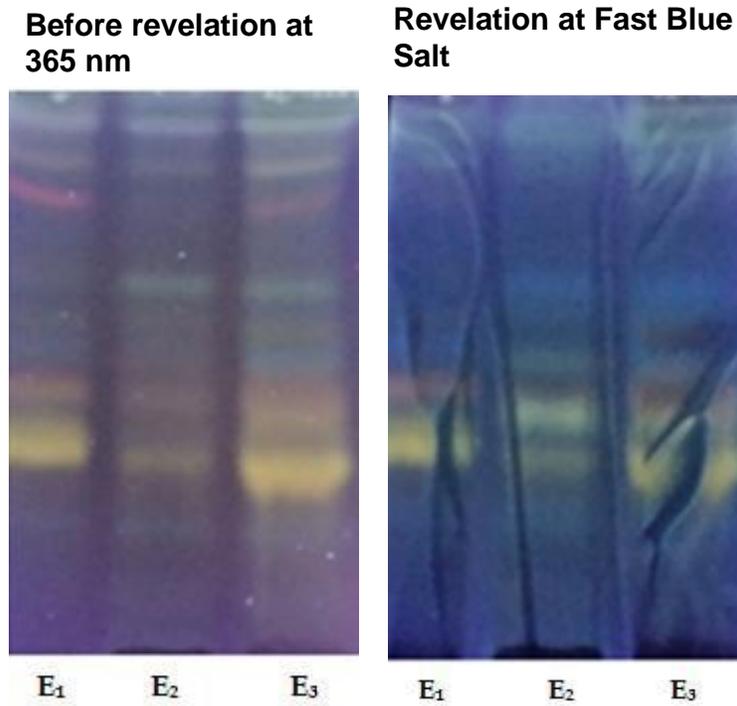


Figure 4. Third chromatogram of TLC result of tannins and coumarins in polar extracts from the stem bark of *H. crispiflorus* A.Rich. Developer: *Fast Blue Salt B* reagent E₁: EtOH; E₂: EtOH-H₂O; E₃: aqueous.

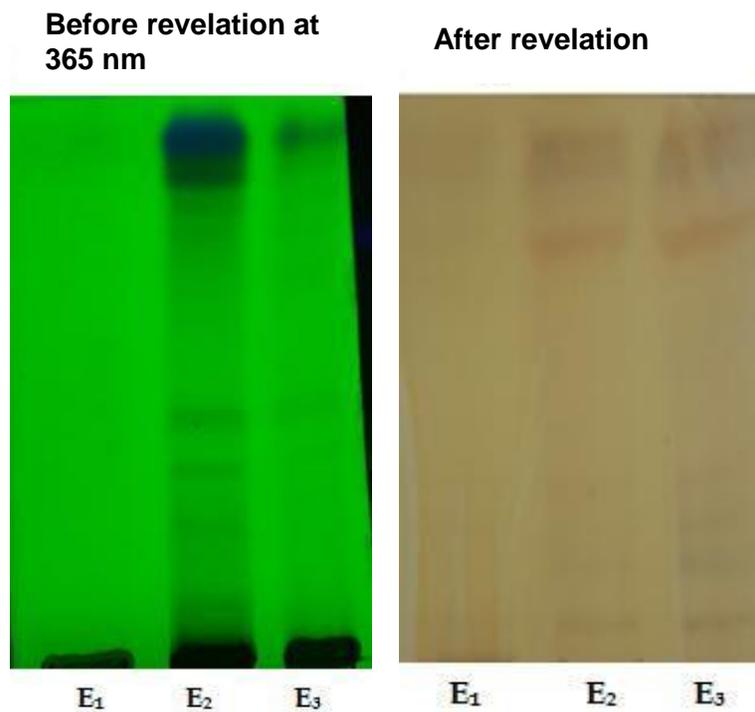


Figure 5. Fourth chromatogram of TLC result for alkaloids from polar extracts from the stem bark of *H. crispiflorus* A.Rich. Developer: Dragendorff E₁: EtOH; E₂: EtOH-H₂O; E₃: aqueous.

Table 2. Summary of results of spectrophotometric assays performed on polar extracts of *H. crispiflorus*.

Organ	Extracts	Total polyphenols (µg Eq AG/g MS)	Total flavonoids (µg Eq Quer/g MS)	Condensed tannins (µg Eq Cat/g MS)
Bark of <i>H. crispiflorus</i>	EtOH (100%)	700.69 ± 2.19	439.27 ± 5.96	165.67 ± 1.51
	EtOH-H ₂ O (70%)	929.11 ± 4.25	488.21 ± 3.96	151.74 ± 1.31
	EtOH-H ₂ O (50%)	955.11 ± 0.76	779.85 ± 7.93	166.33 ± 1.08
	Aqueous	715.78 ± 0.80	210.67 ± 1.92	42.28 ± 0.76
Overall average		823.38 ± 2.00	479.50 ± 4.94	131.50 ± 1.16

Values are the mean of three replicates ± SEM

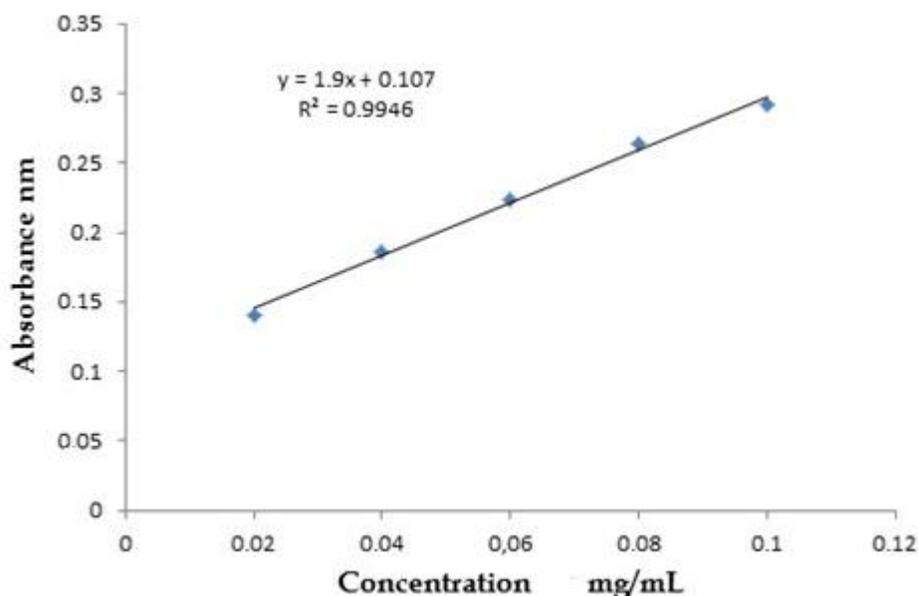


Figure 6. Gallic acid calibration curve for the phenols.

condensed tannins in the polar extracts of *H. crispiflorus* was carried out using separate colorimetric methods (Folin-Ciocalteu, aluminium chloride and sulphuric vanillin). Quantitative analyses of phenolics, total flavonoids and condensed tannins in the different crude extracts were determined from the linear regression equations of the calibration curves, expressed as mg gallic acid equivalent, mg quercetin equivalent and mg catechin equivalent per gram of dry matter, respectively (Figures 6 to 8).

The results of the quantitative analyses by UV-visible spectrophotometry of the different crude extracts of *H. crispiflorus* studied are shown in Table 2. It was observed that the 50% hydroethanolic and 80% hydroethanolic extracts of the stem barks of this species are quantitatively richer in phenolic compounds, namely polyphenols (955.11 ± 0.76 to 929.11 ± 4.25 µg Eq GA/g Ms) and flavonoids (779.85 ± 7.93 and 488.21 ± 3.6 µg Eq Quer/g Ms), respectively. Tannins were present but in moderate amounts ranging from 166.33 ± 1.08 to 165.67

± 1.51 µg Eq Cat/g Ms in the 50 and 80% hydroethanolic extracts, respectively. It was found that this plant has high levels of total polyphenols compared to flavonoids, both in the 50 and 80% hydroethanolic extracts.

These results confirm the high level of phenolic substances present in *H. crispiflorus*, as revealed by the tube and TLC reactions.

Scavenging of the free radical DPPH

Of the four (04) *H. crispiflorus* extracts studied, only the aqueous extract was more active with an inhibition percentage of around 39.85% in the bark (Figure 9).

Antioxidant activity in 96-well microplates gave inhibition percentages of around 39.85, 19.47, 17.94, and 5.79% for the aqueous, ethanol-water (50%), ethanol-water (80%), and ethanol (100%) extracts, respectively. It should be noted that the aqueous extract of *H. crispiflorus* showed greater anti-free radical activity than

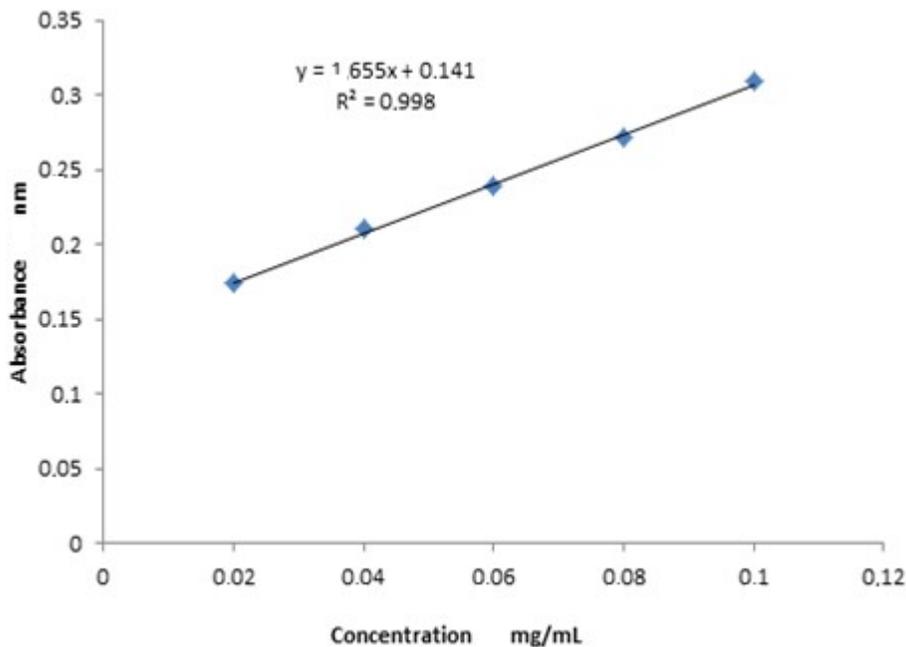


Figure 7. Quercetin calibration curve for the determination of total determination of total flavonoid.

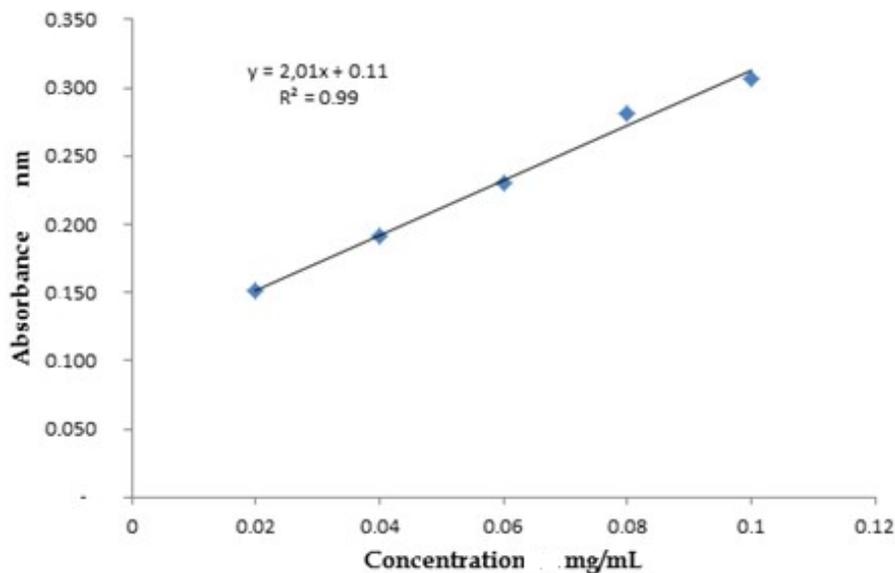


Figure 8. Catechin standard line for the determination of condensed tannins.

the other extracts. However, the four (04) extracts studied had a weaker anti-free radical power compared to the reference molecule, ascorbic acid, which had an inhibition percentage of 89.4%.

Previous work by Afua (2016) on the methanolic extract of fresh *H. monopetalus* leaves gave an IC₅₀ value much lower than that of the standard (ascorbic acid).

The difference in the inhibition percentages obtained in each type of extract could therefore be due to the presence and structural nature of the phenolic compounds in these extracts. Furthermore, this low activity indicates that the different polar extracts of *H. crispiflorus* contain few substances that react with the DPPH radical.

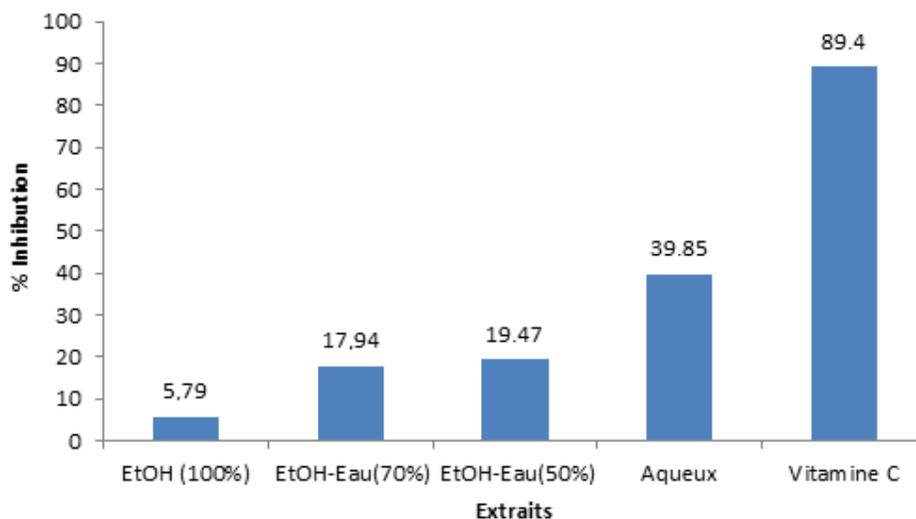


Figure 9. Percentage Inhibition of antioxidant activity of different polar extracts of the plant *H. crispiflorus* A Rich.

Conclusion

The present work involved a phytochemical screening to evaluate the antioxidant activity of *H. crispiflorus* from the Central African Republic, which had not been chemically studied. Tube reaction and TLC analyses identified the following secondary metabolites: flavonoids, anthocyanins, tannins, catechic tannins, anthracene derivatives, quinones, saponosides, reducing sugars, triterpenes, and sterols. Quantitative analysis of the different polar extracts showed that *H. crispiflorus* bark had a high polyphenol and flavonoid content in the hydroethanolic extracts (50 and 80% respectively). The evaluation of the antioxidant activity showed that the hydroethanolic (50 and 80%) and aqueous extracts have a significant inhibition percentage compared to the reference molecule ascorbic acid. Taken together, these results provide scientific justification for the traditional use of the plant studied. However, these results are only a first step in the field of phytochemical research. It would be interesting to continue the phytochemical study of this plant with the aim of separating and isolating the chemical constituents and carrying out biological tests in order to scientifically verify the traditional use of *H. crispiflorus* in the treatment of skin diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abderaman BS, Behanzin J, Ahokpe M, Sezan A (2018). Evaluation of

the toxicity of the ethanolic extracts of the leaves of *Hexalobus monopetalus* on the kidneys and the liver of Wistar rats. International Journal of Engineering Technologies and Management Research 5(11):1-12.

Afua ME (2016). Phytochemical constituents and some biological activities of selected medicinal plants from Ghana. Ph. D thesis, Kwame Nkrumah University.

Akotegnon R, Michodjehoun L, Behanzin J, Houeze E, Sezan A (2018). Impact des extraits éthyliques des feuilles de *Hexalobus Monopetalus* sur le métabolisme protéiques des rats Wistar. International Journal of Multidisciplinary and Current Research 6:2321-2324.

Couvreur TLP, Dagallier MJ, Crozier F, Ghogue JP, Hoekstra P, Kamdem N, Johnson D, Murray N, Sohké B (2022). Flora Cameroon – Annonaceae. PhytoKeys 207:1-532.

Dieng MSI, Fall D, Diatta-Badji K, Sarr A, Sene M, Sene M, Mbaye A, Diatta W, Bassene E (2017). Evaluation de l'activité antioxydante des extraits hydro-ethanoliques des feuilles et écorces de *Piliostigma thonningii* Schumach. International Journal of Biological Chemical Sciences 11(2):768-776.

Dirar AI, Alsaadi DHM, Wada M, Mohamed MA, Watanabe T, Devkota HP (2019). Effets de extraction solvant on total phenolic and flavonoid content and biological activities of extracts from Sudanese medicinal plants. South African Journal of Botany 120:261-267.

Ehiabhi OS, Adeniyi YT, Okechuchwu EB (2016). Assessment of Antiproliferative Potential of *Hexalobus rispiflorus* (Annonaceae). British Biotechnology Journal 15(3):1-7.

Hamisi MM, Stephan AJ, Reiner W, Mayunga HHN (2014). Diprenylated Indole Alkaloids from Fruits of *Hexalobus monopetalus*. Natural Products and Bioprospecting 4(2):101-105.

Kouamé T, Siaka S, Kassi A B, Soro Y (2021). Détermination des teneurs en polyphénols totaux, flavonoïdes totaux et tanins de jeunes feuilles non-encore ouvertes de *Piliostigma thonningii* (Caesalpinaceae). International Journal of Biological Chemical Sciences 15(1):97-10.

Kouchadé S, Adjatin R, Cossi A, Gbèwonmèdéa H, Akoègninou A (2017). Phytochimiques des plantes médicinales utilisées dans la prise en charge des maladies infantiles au Sud-Bénin. European Scientific Journal 13(3):1857-7881.

LACCEG (2015). Laboratory of Climatology, Cartography and Geographical Studies, Faculty of Letters and Humanities, University of Bangui.

Mahadeva R, Muhammad A, Khamsah M (2016). Phytochemical

- screening, total flavonoid and phenolic content assays of various solvent extracts of Tepal of *Musa paradisiaca*. *Malaysian Journal of Analytical Sciences* 20(5):1181-1190.
- Ngaïssona P, Namkona FA, Koane JN, Tsiba G, Syssa-Magalé JL, Ouamba JM (2016). Phytochemical screening and evaluation of antioxidant activity of polar extracts *Picralima nitida* Stapf. (Apocynaceae) family. *Journal of Pharmacognosy and Phytochemistry* 5(4):198-204.
- Okhale SE, Tijani A, Ezugwu BO (2016). Assessment of Antiproliferative Potential of *Hexalobus crispiflorus* (Annonaceae). *British Biotechnology Journal* 15(3):1-7.
- Onuminya TO, Shodiya OE, Olubiyi OO (2017). Comparative proximate and phytochemical analysis of leafy vegetables in Lagos, Nigeria. *NJPAS* 30(3):3097-3103.
- Rachedi-Ali F, Meraghni S, Touaibia N, Sabrina M (2018). Analyse quantitative des composés phénoliques d'une endémique algérienne *Scabiosa Atropurpurea* sub. *Maritima* L. *Bulletin de la Société Royale des Sciences de Liège* 87:13-21.
- Rahul R, Arrivukkarasan S, Anhuradha S (2022). *In Vitro* Antioxidant and Free Radical Scavenging Activity of *Curcuma longa*, *Acorus calamus* and *Camellia sinensis*. *Food and Nutrition Sciences* 13:750-760.
- Tijani A, Mohammed T, Alkali T, Adamu B, Abdurahaman I (2017). Phytochemical analysis, analgesic and antipyretic properties of ethanolic leaf extract of *Vernonia amygdalina* Del. *Journal Herbmed Pharmacology* 6(3):95-99.
- World Health Organization (WHO) (2012). *Strategy for Traditional Medicine 2007-2011*. Geneva.