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Research article

Phytochemical study and anti-sickling, anti-inflammatory, antioxidant and cytotoxic activities of *Uvariodendron molundense* (Annonaceae) bark

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Abstract: The present study showed that the bark of *Uvariodendron molundense* contains characteristic histological elements such as fibre fragments, epidermal cell fragments, hexagonal isodiametric epidermal cells, hexagonal epidermal cells, polyhedral cells and crystalline fibre fragments. It also contains tannins, saponosides, flavonoids, phenolic acids, iridoids, anthocyanins, anthrones, anthraquinones and terpenes. The total polyphenol content was 419.514±1.666 mg EAG g^{-1} extract and the total flavonoid content was 2.510±0.348 mg EQ g^{-1} extract. The results also shown that the anti-free radical activity of the extract derived from percolation is greater than that of the decoctation extract and that *U. molundense* bark are not cytotoxic (%Hemolysis <50 at 1000 µg ml^{-1}). These leaves have anti-inflammatory properties. However, this activity is low for the percolation-derived extract (%I = 49.287±7.180%) and average for the decoction derived extract (%I = 51.928±5.882%). Both decoction and percolation-derived extracts of *Uvariodendron molundense* species displayed good antisickling properties *in vitro*. The results of molecular docking indicate that Eugenol (which is the major component of essential oil of *Uvariodendron* genus) forms two hydrogen bonds with haemoglobin S [F103(F)O; G107(F)CA], three hydrogen bonds with nuclear factor NF-kB [E346(F)0E2; Y379(F)O; Y379(F)N] and a single hydrogen bond with myeloperoxidase [M(A)O]. Based on molecular modelling, we can therefore hypothesise that this medicinal plant acts by inhibiting haemoglobin S, nuclear factor NF-kB and myeloperoxidase respectively. Study of the interaction of this compound/ligand (Eugenol) with three receptors: haemoglobin S (ΔG = -5.91±0.21 kcal), NF-kB (ΔG = -4.98±0.22 kcal) and Myeloperoxidase (ΔG = -5.85±0.29 kcal) indicates the formation of a thermodynamically stable complex (ΔG <0). A more in-depth phytochemical study of *U. molundense* bark is therefore desirable in order to identify the biologically active compounds.
Keywords: *Uvariodendron molundense* - Eugenol, - Haemoglobin S - NF-kB - Myeloperoxidase.

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INTRODUCTION

Sickle cell disease or SS anaemia is a haemoglobinopathy characterised by the presence of abnormal

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haemoglobin S (Hb S) in the blood of patients. It is an autosomal recessive genetic disease with highly variable phenotypic expression (Ngbolua 2019, Cheikhouna 2021, Masengo *et al.* 2021a). The presence of Hb S is the result of a missense point mutation on the sixth codon of the βS gene on chromosome 11 in humans. The replacement of the nitrogenous base adenine by thymine (GAG→GTG) results, in the ß globin chain, in the substitution of glutamic acid in position 6 by valine, a hydrophobic amino acid (Girot *et al*. 2003, Cheikhouna 2021).

In Africa, sickle cell disease is a public health problem, with a very high prevalence rate in Central and West Africa (20–40% of subjects are carriers of the sickle cell trait). Epidemiological data indicate that in the Democratic Republic of Congo (DRC), more than 2% of the population is affected, i.e. almost one and a half million people (Mpiana *et al*. 2007, Tshilanda *et al.* 2015). Sickle cell disease is characterised physiologically and biochemically by functional asplenia and dysfunction of the pentose phosphate shunt, which promotes sepsis and the production of free radicals, leading to a permanent inflammatory state in homozygous sickle cell patients (Dembele 2020). This is why, in addition to the intrinsic causes, the management of this genetic disease must take into account infectious and inflammatory aspects and oxidative stress. Numerous therapeutic strategies have been developed to combat this haemoglobinopathy, but they do not bring the desired success and are either expensive or toxic, and are not accessible to low-income populations (Mpiana *et al*. 2007).

Traditional medicine is a relevant alternative, as over 80% of the population use it for their primary healthcare (Mpiana *et al*. 2010a, b, c, Mpiana *et al*. 2011, Masengo *et al*. 2021b). These medicinal plants are rich in bioactive secondary metabolites that form a balanced chemical complex. However, it should be noted that abusive and uncontrolled use of these plants can often expose consumers to side effects despite the presence of bioactive metabolites (Gueyraud *et al*. 2019).

The doses used for the various traditional treatments are imprecise, which is why it is essential to assess the efficacy and toxicity of medicinal plant extracts to prevent the risk of therapeutic accidents. It is well-established that the DRC is a global reservoir of medicinal plants that play a key role in the management of sickle cell disease (Mpiana *et al*. 2016, Ngbolua *et al*. 2019, Djolu *et al*. 2021).

Thus, the use of these plant genetic resources for various health problems is not only a choice, but also because of poverty and the high cost of modern medicines (Ngbolua *et al*. 2011a, b). The objectives of the present study include identifying the chemical groups and assessing the anti-sickle cell, the anti-inflammatory, anti-free radical and cytotoxic activity of *Uvariodendron molundense* (Diels) R.E.Fr. bark. This study is of obvious interest because, if the pharmacological properties of the bark from this bio-resource are validated, it will lead to the development of an anti-sickle cell drug, the marketing of which could promote the effective implementation of the Nagoya protocol on ABS (Access and Benefit Sharing from the Exploitation of Plant Genetic Resources) in the DRC.

MATERIALS AND METHODS

Material

The plant material used in this study consisted of *Uvariodendron molundense* stem bark. Blood samples were taken from sickle cell patients attending the Centre de Médecine Mixte et d'Anémie SS "CMMASS" in Kinshasa/Kalamu. The blood sample used for the cytotoxicity test was taken from a healthy volunteer. Hen eggs (*Gallus gallus*) were used as the source of ovalbumin in the anti-inflammatory test.

Methods

i. *Collection, processing and packaging of samples*: The *Uvariodendron molundense* leaves used in this study were collected from the village of Mbui (Latitude: 4° 16' 16" N; Longitude: 21° 7' 23" E; Altitude: 400 m above sea level), in the Mobayi-Mbongo Territory, North Ubangi Province. Figure 1 shows the geographical location of Mbui village in the province of North Ubangi in the DRC.

After harvesting the plant material, the samples were dried for approximately one month in the shade (Fig. 1A) and then ground to powder (Fig. 1B) using an HSMFM electric grinder.

ii. *Micrography of powders*: Optical micrography of the powders was carried out according to the following procedure :

- Place 2 to 3 drops of Steinmetz reagent on a slide ;
- Carefully drop a small quantity of powder, removed with a fine spatula, into the reagent ;
- Cover with a cover slip and press lightly to homogenise the preparation ;
- Absorb any burrs and wipe the underside of the slide with a paper towel.
- The micrographic examination (enlargements, etc.) can now be carried out.

The outer surface of the slide must first be cleaned of all traces of the reagent or powder to be examined. Care must also be taken to prepare the slides very lightly, to distribute the tissues evenly and avoid overlapping (Inkoto *et al*. 2021, Ngbolua *et al*. 2021b).

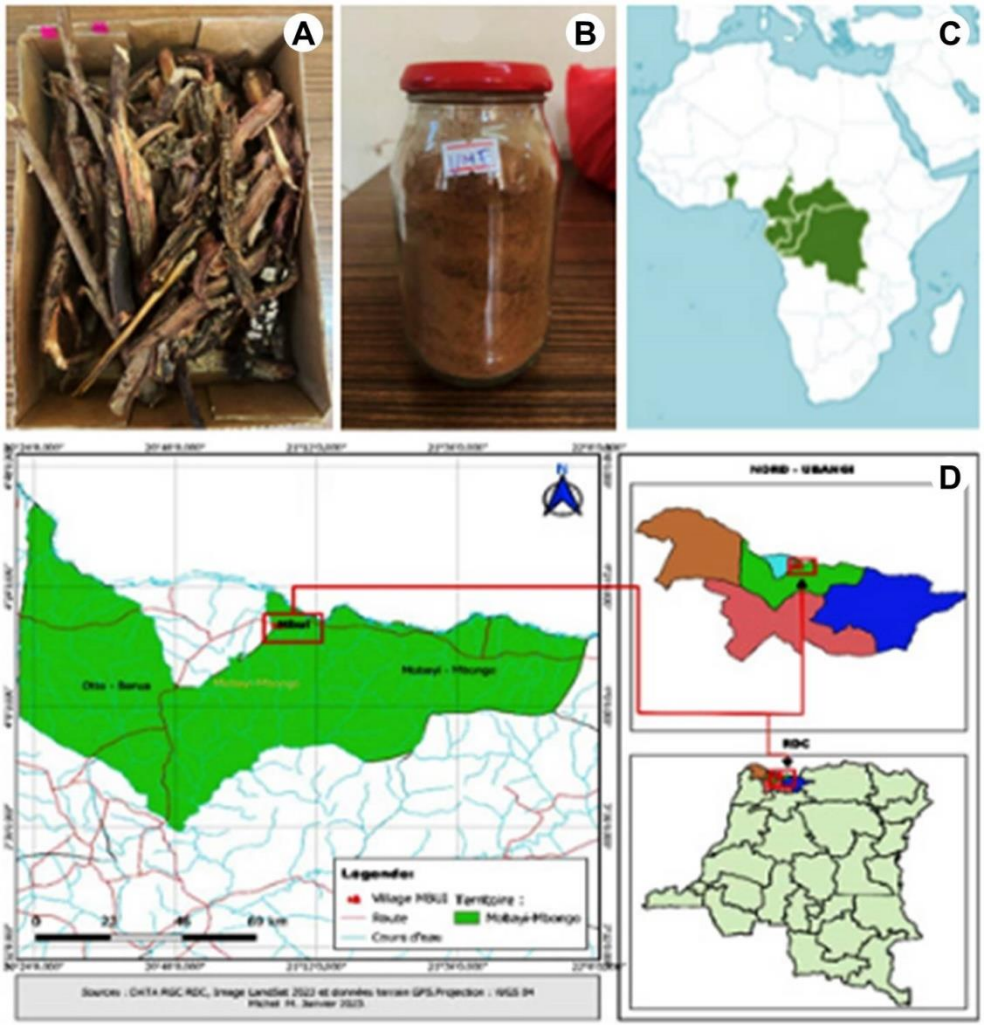

Figure 1. Geographical location of Mbui village and surrounding area [Province of Nord-Ubangi, DRC: Figure 1C (© : www.powo.org) & figure 1D].

iii. *Qualitative phytochemical analysis*:

- Preparation of extracts of *U. molundense*

The extracts were prepared by dissolving the plant powders in a suitable solvent at a ratio of 1:10 (w/v).

- Solution reactions

The characterization reactions were carried out in tubes (solution tests). This is a qualitative analysis based on colouring and/or precipitation reactions.

- Research into total tannins

A few drops of a 1% ferric chloride ($FeCl_3$) solution were added to 2 ml of the solution obtained in the general flavonoid test. A dark blue, black or green colour indicates the presence of tannins (Mpiana *et al*. 2007).

- Alkaloid research

One gram of drug is placed in a wide-necked Erlenmeyer flask and a few drops of 5% HCl added to 5 ml of distilled water. The flask is sealed and left to macerate for 24 hours, stirring occasionally. Filter through cotton wool. Add 5 drops of Mayer's reagent (1.36 g of $HgCl_2$ and 5 g of KI in 100 ml of water) to 1 ml of filtrate. For the Mayer test, take 100 µl of filtrate and add 1 drop of Mayer's reagent. The presence of alkaloids is indicated by the formation of a white precipitate (Mpiana *et al*. 2007).

- Saponosides research

Saponins are highlighted by the foam index, which is given by the degree of dilution of an aqueous decoction of the drug which, under certain conditions, gives persistent foam. In a 500 ml conical flask containing 100 ml of boiling water, introduce 2 g of drug powder. Maintain a moderate boil for 30 minutes.

Filter and adjust the volume to 100 ml after cooling. Measure 1, 2, 3, 4, and 10 ml of decoction successively in a set of 10 test tubes, each with a height of 16 cm and a diameter of 16 mm. Adjust the volume of each tube to 10 ml using distilled water.

Shake each tube lengthwise for 15 seconds: two shakes per second, after stoppering with your thumb. Leave to stand for 15 minutes and measure the height of the foam. If it is less than 1 cm in all the tubes, the index is less than 100 (negligible). If it is 1 cm in one of the tubes, the dilution of the drug in this tube is the foam index (Mpiana *et al*. 2007).

iv. *Phytochemical screening by Thin Layer Chromatography* (Ngbolua *et al*. 2021b).

- Flavonoids and phenolic acids research

a. Sample preparation

1 g of pulverised drug was extracted with stirring in 5 ml of methanol for 10 minutes, and 10 ml of filtrate was used for TLC analysis.

b. Chromatographic conditions

- ✓ Stationary stage : Silicagel F $_{254}$
- ✓ Phase mobile 1 : ethyl acetate-formic acid-methanol-water (40: 1:5:4)

Control: Chlorogenic acid and Rutin 1 mg ml^{-1} (methanol) deposit: 10 µl

Mobile phase 2: dichloromethane-formic acid-acetone (40:5:10)

Control: Gallic acid, Quercetin, Kampferol and Rosmarinic acid: 1 mg ml^{-1} (methanol) deposit: 10 µl

Detection: the chromatogram, once developed, is observed under UV at 254 and 366 nm, then sprayed with Neu's reagent (DPBAE/PEG) and observed under UV at 366 nm. The presence of flavonoids is indicated by the presence of fluorescent spots of various colours (yellow-orange-green) that vary according to the structure of the compounds detected. Blue fluorescence is often due to phenolic acids.

- Iridoids research

a. Preparing samples :

The solution prepared for the flavonoid test is used: 10 µl deposits.

b. Chromatographic conditions

- Stationary stage: Silicagel F $_{254}$
- Phase mobile**:** ethylacetate-methanol-water (50 : 6. 75: 5)
- Revelation: 5% sulphuric acid in ethanol.
- Heated for 10 minutes at 100°C.
- True iridoids are coloured, while other terpenes are coloured black.

- Anthocyanin research

a. Preparing samples

The solution prepared for the flavonoid test is used: 10 µl deposits.

b. Chromatographic conditions

- Stationary stage: Silicagel F $_{254}$
- Mobile phase: ethyl acetate-formic acid-water (50:5:20)
- After mixing in a separating funnel, the lower phase is removed and the upper phase is used as the mobile phase.
- Control: D-catechine.
- Revelation: Vanillin phosphoric acid.
- Heat for 10 minutes at 100°C.
- Anthocyanins give pink colouring

- Anthraquinones (hétérosides anthracéniques)

a. Preparing samples

We use the solution prepared during the flavonoid test

b. Chromatographic conditions

- Mobile phase: ethyl acetate-methanol-water (50 : 6.75 : 5)
- Revelation: UV observation at 254 and 366 nm.
- Spraying with 10% ethanolic KOH.
- Anthraquinones are coloured red and fluoresce red at 366 nm, while anthrones (aloin) fluoresce yellow.

- Terpenes

a. Sample preparation

1 g of pulverised drug is extracted with stirring by 6 ml of ethyl acetate for 15 minutes (10 µl deposit).

b. Chromatographic conditions

- Stationary stage: Silicagel F_{254}
- Phase mobile: Ethyl toluene acetate (27:3)
- Control: Thymol, menthol, oleanic acid: 1 mg ml^{-1} (methanol) deposit: 10 µl
- Revelation: Sulphuric vanillin.
- Heat for 10 minutes at 100°C.
- Terpenes give different colours with this reagent.

- Coumarins

a. Sample preparation

The solution prepared for the terpene test is used: 10 µl deposit.

b. Chromatographic conditions

- Mobile phase: toluene-ether (1 : 1, saturated with 10% acetic acid).
- Mix 10 ml toluene, 10 ml ether and 10 ml 10% acetic acid in a separating funnel. The lower phase is discarded and the upper phase used as the mobile phase.
- Revelation: Observation under UV at 254 and 366 nm.
- Sprayed with 10% ethanolic KOHPhase mobile : toluène-éther (1 :1, saturé avec l'acide acétique 10%).
- Coumarins produce a blue fluorescence.

v. *Determination of some secondary metabolites* (Ngbolua *et al*. 2021a,b, Djolu *et al*. 2023)

- *Dosage of total polyphenols*

The total polyphenol content of our extracts was determined using the Folin-Ciocalteu method. Briefly, 10 mg ml^{-1} of each extract was diluted in 80% methanol to obtain a solution of 1 mg ml^{-1} for each extract. We then prepared a reaction mixture for each extract consisting of 0.5 ml extract, 5.0 ml distilled water and 0.5 ml Folin-Ciocalteu reagent. After three minutes, we added 1.0 ml of a saturated 20% Na_2CO_3 solution. The mixtures thus prepared were stirred and incubated at laboratory temperature in the dark for one hour. The absorbances were read with a spectrophotometer at 725 nm. Each assay was repeated three times. The quantity of total polyphenols was expressed in mg gallic acid equivalents (GAE) g^{-1} dry extract using the following equation:

$$y = 0.0037x + 0.0218;\ R^2 = 0.9899$$

Where x is absorbance and y is gallic acid equivalent (mg g^{-1})

The calibration line for the determination of total polyphenols is shown in the figure below:

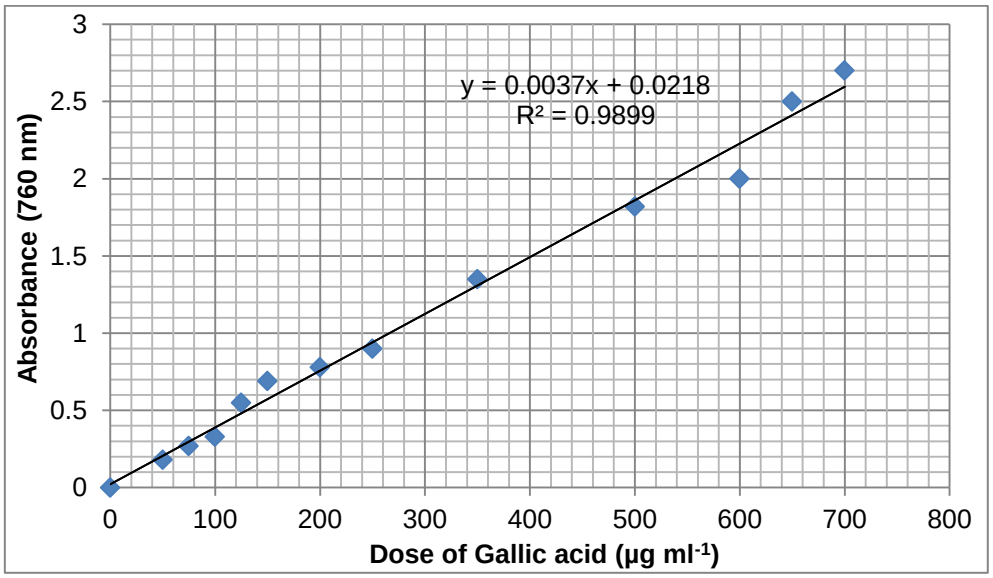

Figure 2. Calibration line for the determination of total polyphenols (Djolu *et al*. 2023).

- *Determination of total flavonoids*

We estimated the total flavonoid content of our extracts using a spectrophotometric method. Aluminium trichloride forms a yellow complex with flavonoids, absorbing at 415 nm. The reaction mixture contained 1 ml of methanolic solution (80%) of each of the extracts at a concentration of 25 mg ml^{-1} and 1 ml of 2% $AlCl_3$ (dissolved in methanol) and was shaken well. After one hour's incubation at laboratory temperature and protection from light, absorbances were measured using a spectrophotometer at 415 nm. Mixtures were prepared in triplicate for each analysis and the mean value was used. For the preparation of the blank, we used 1 ml methanol (80%) and 1 ml $AlCl_3$ (2%). The flavonoid content of the extracts was expressed as mg quercetin equivalent (QE) g^{-1} of corresponding dry extract using the equation from the calibration line:

$$y = 0.0542x-0.0367; R^2 = 0.9874$$

Where x is absorbance and y is quercetin equivalent (mg g^{-1}).

The calibration line for the determination of total flavonoids is shown in the figure below:

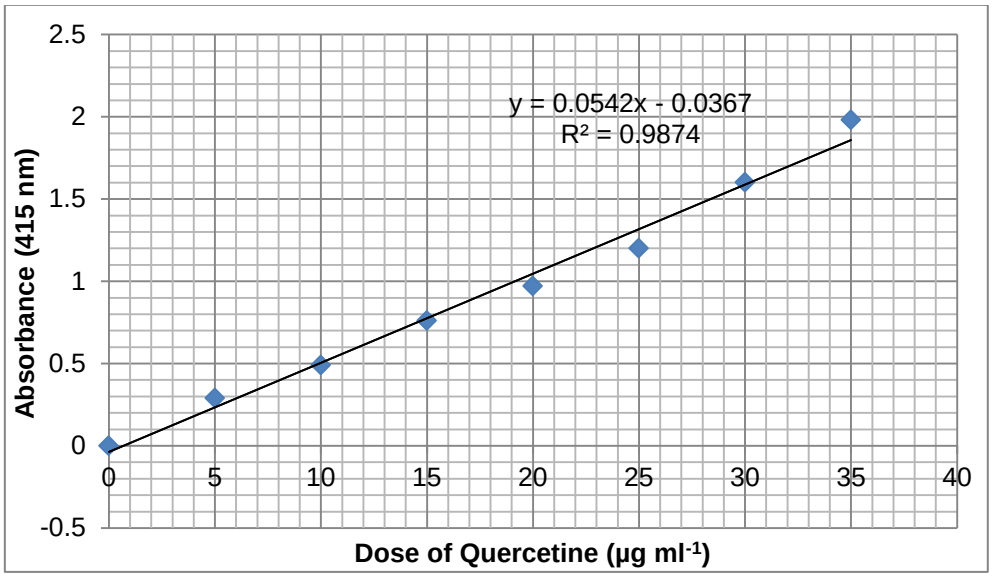

Figure 3. Calibration line for the determination of total flavonoids (Djolu *et al*. 2023).

- *Assessment of anti-free radical activity*

Extracts preparation

- Two types of extracts were used to assess pharmaco-biological properties: the decoctate and the percolate.
- These extracts were prepared according to the following procedures:

Decoction

- Weigh out 10 g of plant powder
- Add 100 ml of water
- Bring the mixture (water + powder) to the boil for 3 to 5 minutes
- Filter then evaporate to dryness on a rotary evaporator
- Place the extract in an oven for 24 hours
- Weigh and store the extract obtained at between 4 and 8 °C

Percolation

- Wet 10 g of powder with the Methanol/Dichloromethane mixture (1:1) (extraction solvent) for 15 minutes
- Place the wetted powder in the percolator (capacity: 100 ml)
- Add 10 to 20 ml of the solvent and leave to macerate for 48 hours
- Allow the percolate to run off drop by drop at a slow speed
- Replenish the solvent until it has been used up (final volume at least 200 ml of percolate)
- Concentrate the dry percolate in a rotary evaporator
- Weigh and store the dry extract obtained at between 4°C and 8°C.

- *Preparation of samples for analysis*
  - Dissolve 10 mg of dry extract from each sample in 1 ml of methanol for the organic extracts and

in 1 ml of the DMSO-Water mixture (1:1) for the aqueous extracts (solution A: 10 mg ml^{-1}).

- Dilute to obtain the following concentrations: 0.5 mg ml^{-1}, 0.4 mg ml^{-1}, 0.3 mg ml^{-1}, 0.2 mg ml^{-1} and 0.1 mg ml^{-1}.

▪ *Test at ABTS*

The principle of this test is based on the reaction of ABTS (2,2'-azino-bis-3ethylBenz-Thiazoline-6-Sulphonic Acid) with potassium or sodium persulphate ($K_2S_2O_8$ or $Na_2S_2O_8$), which forms the cationic radical ABTS with a blue to green colour. The addition of antioxidants reduces this radical and causes the mixture to discolour. The decolourisation of the radical, measured spectrophotometrically at 734 nm, is proportional to the antioxidant concentration.

▪ *Preparing ABTS radical*

- Dissolve 20 millimoles of the ABTS radical in 500 µl of distilled water: solution A
- Dissolve in 500 µl of distilled water a quantity of potassium persulphate ($K_2S_2O_8$) corresponding to 10 millimoles : solution B
- Mix solutions A and B in equal volumes and keep the mixture protected from light for 12 to 16 hours: stock solution of the ABTS radical.
- Dilute the stock solution of the radical with methanol as many times as necessary to obtain an analysis solution with an absorbance of between 0.6 and 0.8.

▪ *Between sample and radical*

- In a test tube, place 20 µl of methanol with 1980 µl of the ABTS radical solution: control solution (3 repetitions).
- In a test tube, place 20 µl of the sample solution for each concentration level (3 repetitions), add to the solution 1980 µl of the ABTS radical analysis solution.
- Leave to incubate in the dark for 30 minutes.

▪ *Absorbance reading at 734 nm*

Take successive readings of the solutions (3 replicates) on the spectrophotometer at 734 nm : the negative control (methanol) and the sample solutions.

▪ *Determination of radical inhibition power*

The percentage inhibition of the $ABTS^{\bullet+}$ radical by the drug is determined using the following formula:

$$\% \text{ Inhibition} = [1 - (A_x/A_c)] \times 100$$

A_x: the absorbance of the $ABTS^{\bullet+}$ radical in the presence of the extract

A_c: absorbance of $ABTS^{\bullet+}$ (control solution)

vi. *DPPH radical assay*

This method is based on the degradation of the DPPH radical (2,2 DiPhenyl-1- PicrylHydrazyl). The DPPH radical is a violet-coloured radical, and the addition of antioxidants reduces this radical and causes the mixture to discolour; this discolouration of the radical, measured by spectrophotometer at 517 nm, is proportional to the antioxidant concentration.

▪ *Preparing the DPPH radical*

- Dissolve 0.25 mg of DPPH in 200 ml of methanol.
- Protect the solution from light for at least one hour

▪ *Contact between sample and radical*

- In a test tube, place 20 µl of methanol with 1980 µl of the DPPH radical solution : control solution (3 repetitions).
- In a test tube, place 20 µl of the sample solution for each concentration (3 replicates), add to the solution 1980 µl of the DPPH
- Leave to incubate in the dark for 30 minutes.

▪ *Absorbance reading at 517 nm*

Take successive readings of the solutions for each concentration (3 repetitions) on the spectrophotometer at 517 nm: the negative control (methanol) and the sample solutions.

▪ *Determination of radical inhibition power*

The percentage inhibition of the DPPH radical by the drug was determined using the following formula :

$$\% \text{ of inhibition} = [1 - (A_x/A_c)] \times 100$$

A_x: the absorbance of the DPPH radical in the presence of the extract

A_c: absorbance of DPPH radical (control solution)

vii. *Cytotoxicity assay*

- Test for qualitative cytotoxicity

The principle of this test is based on the assessment of membrane damage using red blood cells. The apoptosis-inducing capacity of the plant extract on human erythrocytes was assessed by treating blood samples with 0.1% extract for 1 h at room temperature, and using 0.9% NaCl as a control. Blood smears were then prepared, dried, fixed and stained using the May-Grunwald-Giemsa method. After this, the blood smears were observed under a light microscope (OPTIKA) and images of the red blood cells were taken using an iPhone X brand phone (Prajitha & Thoppil, 2017).

- Quantitative cytotoxicity assay

The test is performed by mixing 1 ml of blood diluted to 2.5% (with 0.9% NaCl: 2.5 ml of whole blood plus 100 ml of physiological solution) with 1 ml of extract (1 mg ml^{-1}). The positive control consists of a mixture of distilled water (1 ml) with diluted blood (2.5% in 0.9% NaCl), while the negative control consists of the physiological solution (0.9% NaCl: 1 ml) with diluted blood (2.5% in 0.9% NaCl: 1 ml). The different mixtures were incubated at room temperature for 30 minutes and then centrifuged at 380g for 5 minutes. The optical density (OD) of the supernatant was read at 540 nm using a UV-visible spectrophotometer. For the organic extract, the negative control: 1 ml 0.9% NaCl plus 1 drop of DMSO. The haemolysis rate is calculated using the following formula:

$$\%H = \frac{\text{Extract} - \text{Negative control (NaCl 0.9\%)}}{\text{Positive control} - \text{Negative control}} \times 100$$

The extract is considered cytotoxic when, at 10 µg ml^{-1}, the haemolysis rate is ≥50% (Ngbolua *et al*. 2011a, b, Gbolo *et al*. 2022).

viii. *Anti-inflammatory test (Thermal denaturation of ovalbumin)*

The assay is performed according to the method described by Kumari *et al*. (2015) with slight modification. The reaction mixture consisted of 200 µl egg albumin, 1600 µl phosphate-buffered saline [PBS: 137 mM NaCL (8.0 g l^{-1}), 2.7 mM KCl (0.2 g l^{-1}), 10 mM Na_2HPO_4 (1.44 g l^{-1}), 1.76 mM KH_2PO_4 (0.24 g l^{-1}); pH 6.8] and 1000 µl of distilled water or extract (250 µg ml^{-1}). The mixture was then incubated at 37°C for 15 minutes and heated to 70 °C for 5 minutes. After cooling, absorbance was measured at absorption wavelengths using a spectrophotometer at 650 and 690 nm. Diclofenac sodium (250 µg ml^{-1}) is used as the positive control, while distilled water is used as the negative control. The experiment was performed in triplicate. The rate of inhibition of thermal denaturation of ovalbumin was calculated using the relationship:

$$Denaturing\ inhibition\ (\%) = \frac{\text{Negative control DO} - \text{sample DO}}{\text{Negative control DO}} x\ 100$$

ix. *Assessing anti-sickling activity*

Stock solutions of plant extracts are prepared by simple dilution to 1 mg ml^{-1}. Successive serial dilutions are carried out to obtain solutions of up to 0.31 mg ml^{-1}. Sickle cell blood (0.5 ml) is first diluted with 2 ml of the extract mixture - $Na_2S_2O_5$ 2% (v/v). Microscopic preparations were made by placing a drop of diluted blood and a drop of the drug on the slide. The solution is covered by a slide and the edges of the slides are covered with supercooled paraffin (candle) to create hypoxia. These different preparations are observed under a light microscope 24 hours later (*Ngbolua et al*. 2013).

x. *Molecular docking*

Molecular docking was carried out as previously described by Murail *et al*. (2021).

- *Ethical issues*

The research protocol for this study was approved by the Ethics Committee of the Biology Department at the University of Kinshasa. The study complied with the principles set out in the Declaration of Helsinki (free consent of respondents, etc.). All the rules of confidentiality and ethics as well as the rules of access and benefit sharing (ABS) linked to the use of plant genetic resources in force in the Democratic Republic of Congo were respected in this study.

RESULTS AND DISCUSSION

Micrography

The results of microscopic analyses of *Uvariodendron molundense* bark powder are shown in figure 4. Microscopic analysis of *Uvariodendron molundense* bark powder revealed the presence of fibre fragments (A),

epidermal cell fragments (B), hexagonal isodiametric epidermal cells (C), hexagonal epidermal cells (D), polyhedral cells (E) and crystalline fibre fragments (F), as shown in figure 4.

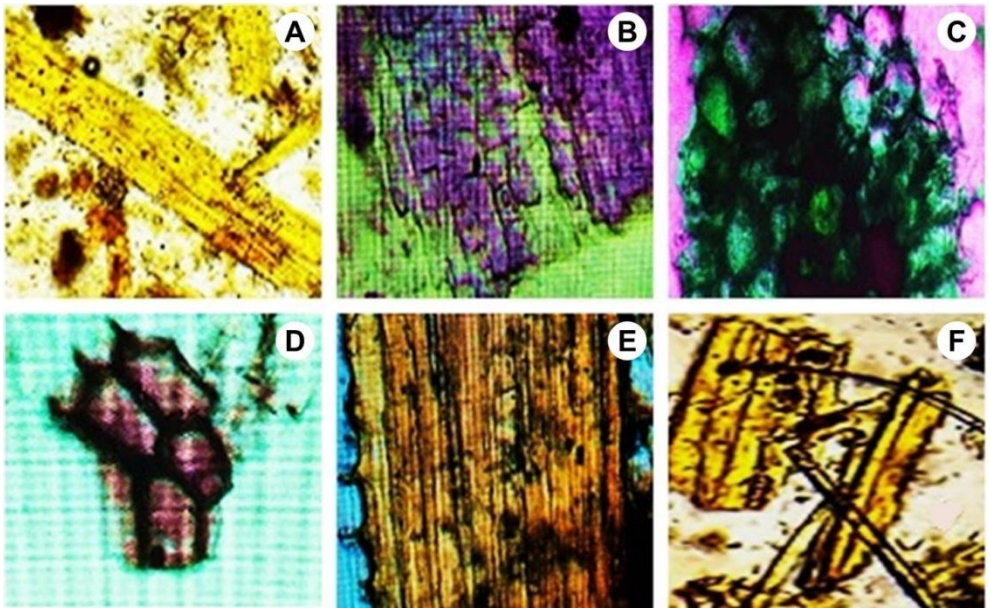

Figure 4. Micrograph of *Uvariodendron molundense* (Diels) R.E.Fr.: A–F, Leaf powder observed under a light microscope (binocular).

Chemical screenings

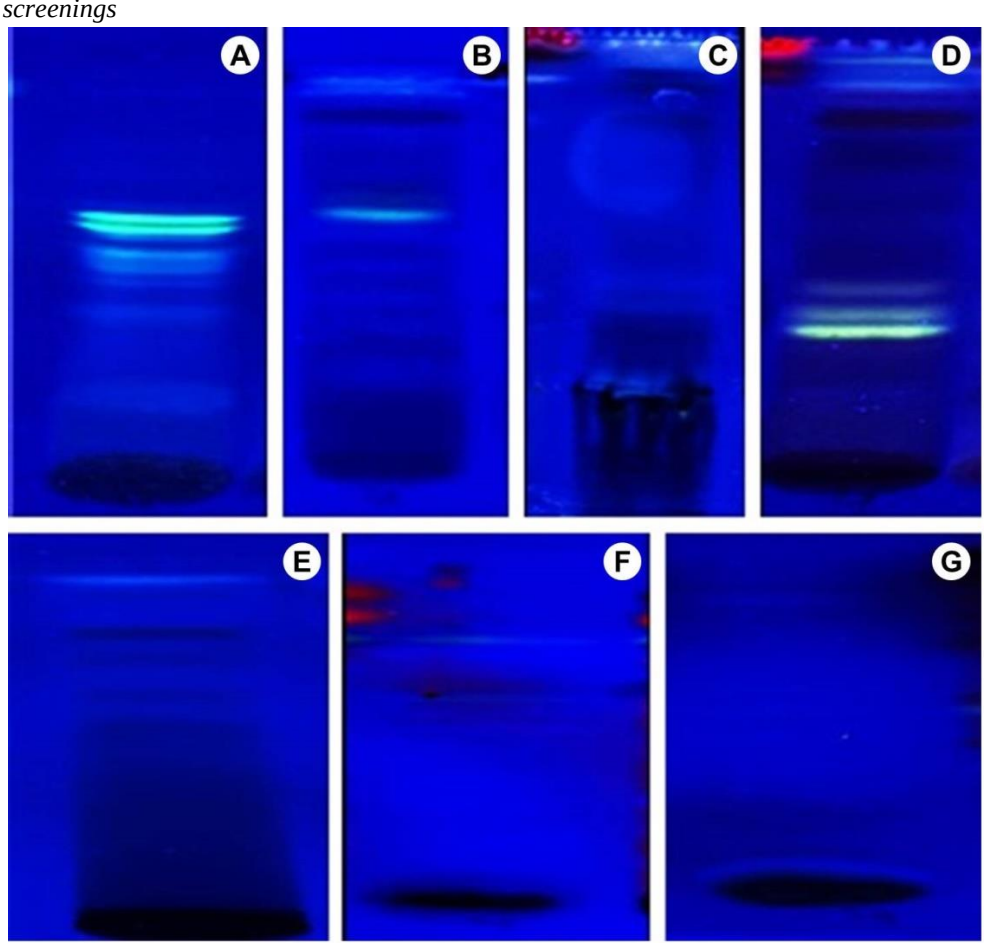

Figure 5. Thin layer chromatographic profile of *Uvariodendron molundense* (Diels) R.E.Fr. bark extracts.

The results of phytochemical screening in solution carried out on the bark of *Uvariodendron molundense* indicated the presence of total tannins and saponosides. However, they did not contain any alkaloids.

These findings are in accordance with the results reported bay Ngbolua *et al*. (2017) and Djolu *et al*. (2023), supporting the identification of tannins and saponins in the plant. Despite literature suggesting the presence of alkaloids in this species, our results differ.

The difference in the chemical composition of this medicinal plant can be explained by the influence of several parameters, in particular extrinsic factors (such as geographical and climatic factors), genetic factors, but also the degree of ripening of the plant and the length of storage, which influence the content of secondary metabolites (Ngbolua *et al*. 2011a, b).

Figure 5 shows the chromatographic profile of methanolic and ethyl acetate extracts of *Uvariodendron molundense*.

Chromatographic analysis revealed the presence of flavonoids (A), phenolic acids (B), iridoids (C), anthocyanins (D) and anthraquinones (E), terpenes (F) and coumarins (G). However, coumarins were not detected in *Uvariodendron molundense* leaves by Djolu *et al*. (2023). Some phytochemical compounds identified in the bark of this plant have interesting pharmaco-biological properties, notably anthocyanins, flavonoids and phenolic acids, whose anti-sickle cell activity in vitro is well established (Mpiana *et al*. 2008, Mpiana *et al*. 2010a, Ngbolua *et al*. 2015, Tshilanda *et al*.2016, Gbolo *et al*. 2022). The therapeutic action of medicinal plants is due to these chemical compounds or secondary metabolites (Lagnika *et al*. 2016).

Quantitative assay of secondary metabolites

The results of the secondary metabolite assay are given in table 1.

Table 1. Total polyphenol and flavonoid content of *Uvariodendron molundense* (Diels) R.E.Fr. bark.

Total polyphenols			Total flavonoids		
Absorbance	**Concentration**	**Average ± Standard deviation**	**Absorbance**	**Concentration**	**Average ± Standard deviation**
1.575	419.784	419.514±1.666	0.126	3.002	
1.581	421.405	mg EAG g^{-1} extract	0.085	2.245	2.510 ± 0.348
1.566	417.351		0.087	2.282	mg EAG g^{-1} extract

Note: EGA g^{-1}: Gallic acid equivalent per gram of dry extract; EQ g^{-1}: Quercetin equivalent per gram of dry extract.

This table shows that extracts of *Uvariodendron molundense* bark have a total polyphenol content of 419.514±1.666 mg EAG g^{-1} extract and flavonoids of 2.510±0.348 mg EQ g^{-1} extract. These compounds are therefore more concentrated in the leaves than in the bark, as indicated by the work of Djolu *et al*. (2023). However, the work of Ngbolua *et al*. (2017) gives lower values than the present study for total polyphenol content (93.44±0.10 mg EQ g^{-1} extract). The presence of these secondary metabolites justifies the use of this plant in traditional medicine. Phenolic compounds such as anthocyanins and flavonoids have anti-sickle cell properties (Mpiana *et al*. 2008, Gbolo *et al*. 2022).

Antioxidant activity

Figure 6 shows the rate of inhibition of the ABTS radical by extracts (percolate) of *Uvariodendron molundense* bark. Figure 6 shows that the rate of inhibition of the ABTS radical is greater than 90%, which indicates that this plant is endowed with very high antiradical activity.

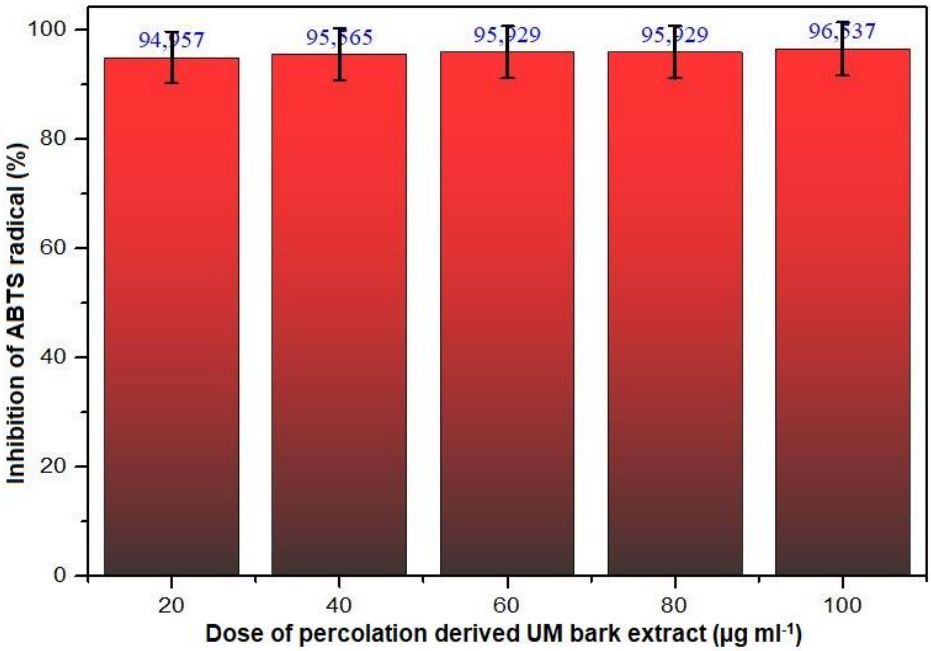

Figure 6. Inhibition of the ABTS radical by *Uvariodendron molundense* (Diels) R.E.Fr. percolation derived extract.

Figure 7 shows the rate of inhibition of the ABTS radical by the decoctate of *Uvariodendron molundense*. Although the decoctate has anti-free radical properties with respect to the ABTS radical, this is low compared with the percolate. The IC_{50} calculated is 42.307 µg ml^{-1}.

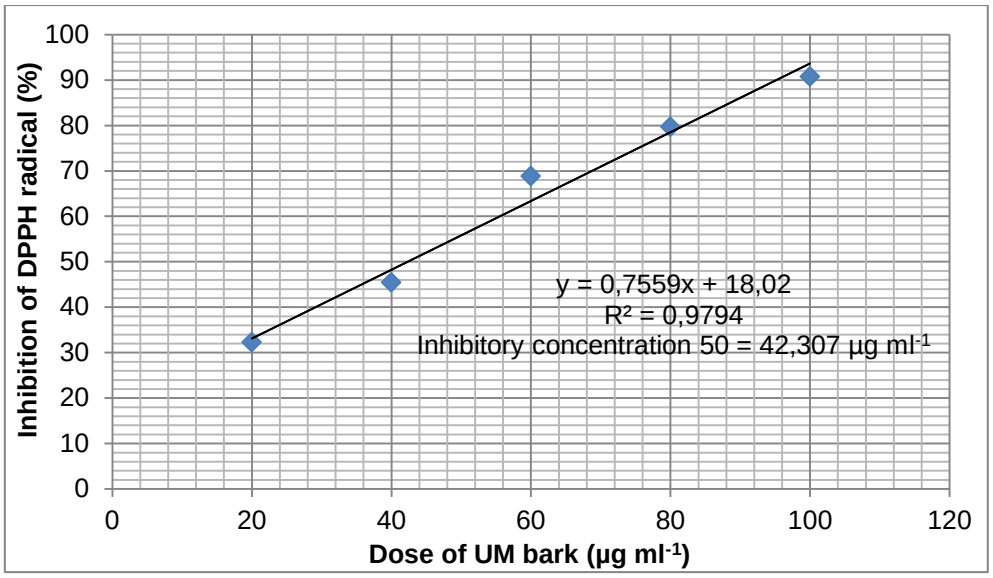

Figure 7. Rate of inhibition of the ABTS radical by the decoction derived extract of *Uvariodendron molundense* (Diels) R.E.Fr.

Table 2 shows the anti-free radical activity of *Uvariodendron molundense* towards the DPPH radical. Comparing these two extracts, we note that the percolate extracts have a greater inhibition of the DPPH radical than the decoctate.

Table 2. Rate of reduction in anti-free radical activity.

Ci (mg ml^{-1})	Cf (µg ml^{-1})	%I Percolation	%I Decoction	Reduction activity (%Δ)
2	20	27.696	11.228	59.460
4	40	32.845	11.512	64.951
6	60	43.009	12.201	71.632
8	80	45.539	13.255	70.893
10	100	48.380	18.322	62.129

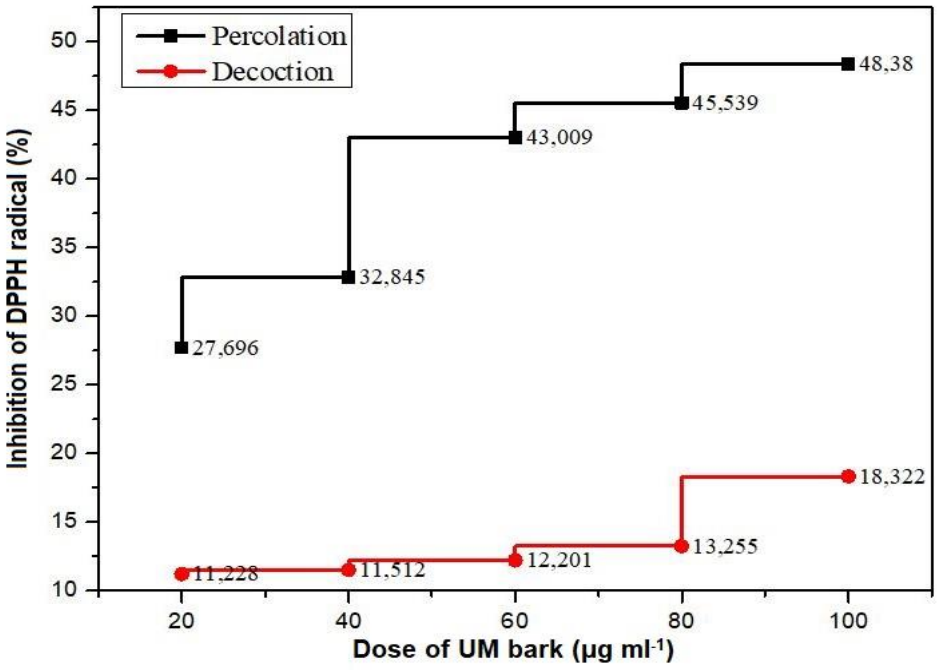

Figure 8. Inhibition of the DPPH radical.

Table 2 shows that the antioxidant activity of the percolate is greater than that of the decoctate with respect to the DPPH radical. However, this activity was low, since at 100 µg ml^{-1}, the inhibition rate of the percolate

was 48.380%, whereas that of the decoctate was only 18.322%, corresponding to a 62.129% reduction in the activity of this plant when exposed to heat (Table 2). As a denaturing agent, heat destroys the active ingredients contained in the bark of this medicinal plant.

Figure 8 shows that inhibition of the DPPH radical by extracts of *Uvariodendron molundense* bark is dose-dependent. The choice of the two radical models is due to the fact that the ABTS radical reacts with both polar and apolar compounds, whereas the DPPH radical only reacts with polar compounds (Heroual *et al*.2020). The results of this work show that our extracts have a reducing effect due to the polyphenolic compounds (Melakhessou 2019). Thus, *U. molundense* bark is a good source of natural antioxidants for combating the oxidative stress generated by chronic diseases such as sickle cell anaemia. Indeed, one of the main characteristics of sickle cell disease is the production of large quantities of free radicals, leading to permanent oxidative stress and the consumption of nitric oxide by oxygen free radicals. Oxidative stress also affects the Fe^{3+}/Fe^{2+} ratio, which is very high in sickle cell cells, and is involved in sickle cell haemolysis. To this end, the antioxidant properties of a plant such as *U. molundense* therefore demonstrate that it can be useful in the management of sickle cell disease (Mpiana *et al*. 2016).

Cytotoxicity

The results of the qualitative cytotoxicity test carried out with the drug based on *Uvariodendron molundense* are shown in figure 9.

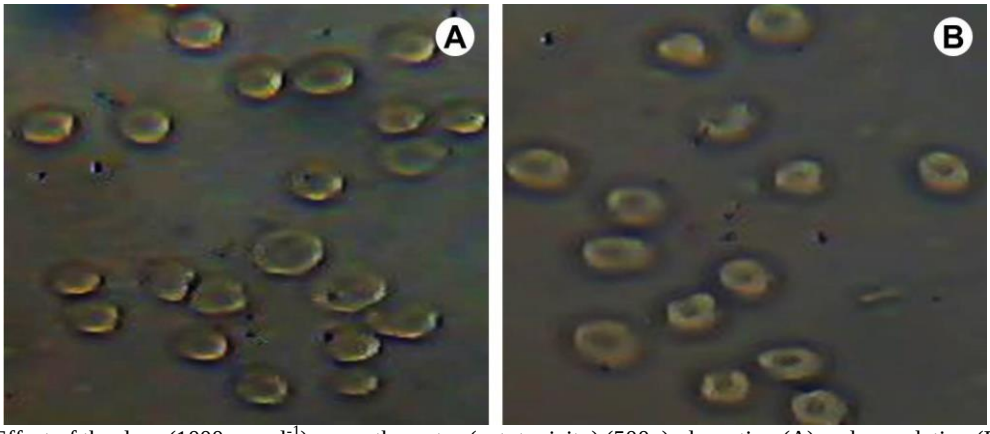

Figure 9. Effect of the drug (1000 µg ml^{-1}) on erythrocytes (cytotoxicity) (500x): decoction (A) and percolation (B).

Figure 9 shows that the decoctate and percolate made from *Uvariodendron molundense* bark are not toxic to erythrocytes. In fact, the erythrocytes retain their biconcave circular shape and a smooth edge, demonstrating the integrity of the biological membrane as indicated by their in vitro phenotype. It has been shown that medicinal plants can pose a toxicity hazard to consumers (Ngbolua *et al*.2011a, b).

The results of the quantitative cytotoxicity test on *U. molundense* bark extracts are shown in figure 10.

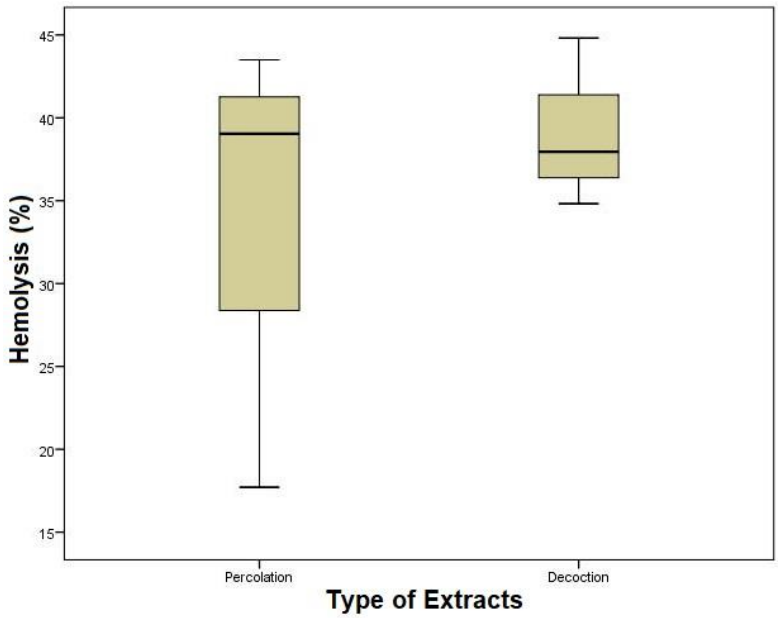

Figure 10. Erythrocyte lysis rate of *Uvariodendron molundense* (Diels) R.E.Fr. bark.

Figure 10 shows that at 1000 µg ml^{-1}, the haemolysis rate of the percolate was 33.414±13.780%, while that of the decoctate was 39.197±5.115%. It should be noted, however, that the difference observed between the two types of extract was not statistically significant (p>0.05). This means that this organ (bark) is not cytotoxic (% Haemolysis <50% at 1000 µg ml^{-1}). This demonstrates the less toxic nature of this plant and guarantees its safety.

Anti-inflammatory action

The results of the effect of drugs on the thermal denaturation of ovalbumin in vitro are shown in figure 11.

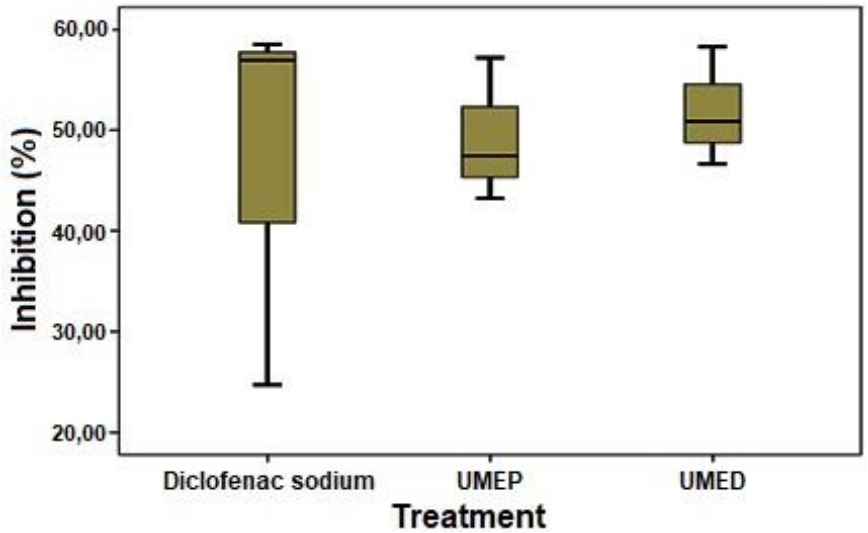

Figure 11. Effect of drugs on thermal denaturation of ovalbumin *in vitro*.

The anti-inflammatory activity of a drug can be assessed on the basis of its rate of inhibition of thermal denaturation of ovalbumin in vitro (%I). Thus, when %I<0 (hyperchromic effect), the product is said to be inactive; when 0<%I<46.734±19.078 (Diclofenac sodium: positive control), the product is weakly active; when 46.734±19.078<%I<75, the product is active and if 75<%I<100, it is very active. This study shows that the decoction of *Uvariodendron molundense* bark (UMED: 51.928±5.882%) and the percolate (UMEP: 49.287±7.180%) are active (Fig. 11).

Sickle cell anaemia is associated with chronic inflammation, which can ultimately lead to failure of vital organs such as the liver, kidneys and heart. This inflammation may be due to abnormal activation of monocytes. The diseased haemoglobin (Hb S) released during haemolysis of the erythrocyte can bind to TLR4 receptors located on the surface of monocytes and trigger inflammation (Dembele 2020). Inflammation is a key component that must be taken into account when defining therapeutic strategies for the symptomatic management of sickle cell disease. It should also be noted that inflammation in sickle cell disease is maintained by sickle cell disease, microbial infections and free radicals. It is clinically characterised by an increase in serum C-reactive protein, alpha 1 acid glycoprotein and a decrease in transferrin levels (Dembele 2020). In the specific case of oxidative stress, the body regards excessive free radicals as pathogenic agents (responsible for intimal hyperplasia of blood vessels) and triggers an inflammatory response in an attempt to eliminate those (Masengo *et al*.2021b). It has been shown that inflammation in sickle cell disease can be modulated by the transcription factor NF-kB via signal transduction pathways involving the p38 MAPK and JNK proteins and eicosanoids (Masengo *et al*.2021b).

In addition to haemoglobin S, 2,3-DPG mutase and the Gardos potassium channel, C-reactive protein, alpha 1 acid glycoprotein, the transcription factor NF-kB, xanthine oxidase/myeloperoxidase and cyclooxygenase are molecular targets of choice for the development of anti-sickle cell drugs. The presence of flavonoids in *Uvariodendron molundense* gives it this anti-inflammatory activity. Flavonoids have anti-inflammatory properties and are capable of modulating immune system function by inhibiting the activity of enzymes that may be responsible for inflammation. They can also modulate monocyte adhesion during atherosclerotic inflammation by inhibiting the expression of inflammatory mediators, and some are capable of inhibiting histamine (Melakhessou 2019).

Anti-sickling activity

Figure 12 shows the morphology of sickle cell erythrocytes untreated and treated with extracts of *Uvariodendron molundense.*

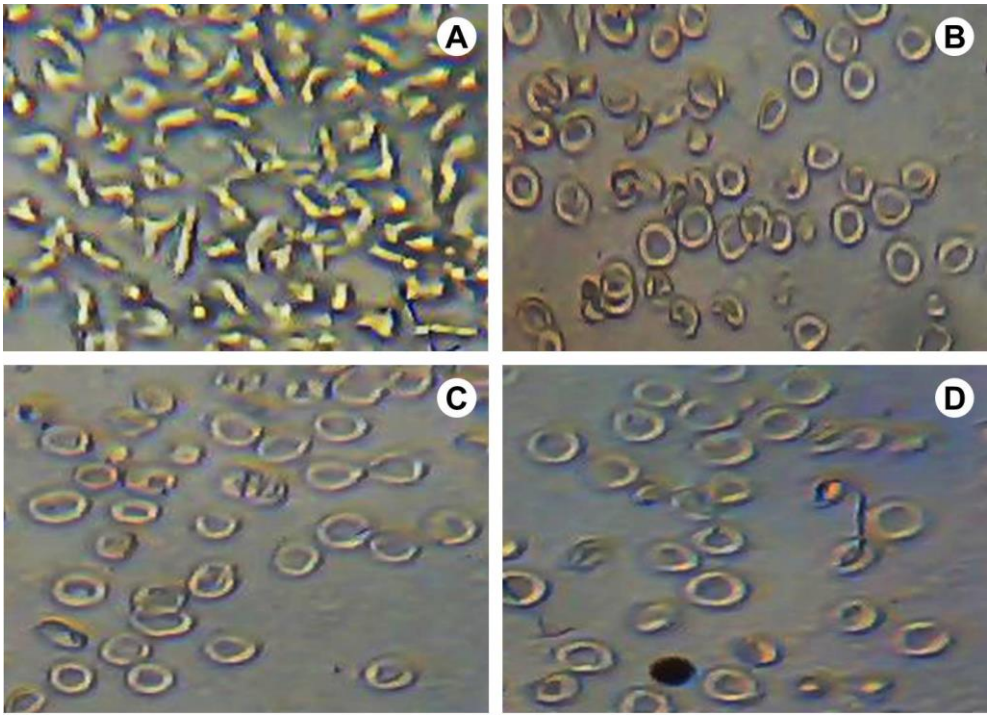

Figure 12. Optical microphotography of control sickle cell blood (A) and treated with *Uvariodendron molundense* (Diels) R.E.Fr. extracts at a concentration of 100 µg ml^{-1}, NaCl 0.9%, $Na_2S_2O_5$ 2%, 500x : Decocted (B) and Percolate (C & D).

Analysis of figure 12 shows that all the cells in the control are sickle-shaped. This confirms that the blood comes from a sickle cell patient. However, in the presence of the extract (decocted/percolate), the sickle cells regained their circular biconcave shape under hypoxic conditions. These results indicate that our active ingredients reduce the ellipsoidality of sickle cells under hypoxic conditions. Our drugs could therefore prevent all the complications associated with sickle cell disease. These results are consistent with those obtained by Ngbolua *et al*. (2017).

The bioactivity observed could be due to secondary metabolites such as anthocyanins, which act by preventing the polymerisation of deoxyhaemoglobin S molecules into tactoids (the polymer responsible for sickle cell disease) as previously reported in the work of Gbolo(2022), Mpiana *et al*.(2016),Ngbolua *et al*.(2017).

Essential oils have also been shown to have anti-sickle cell and anti-free radical properties (Mbula *et al*.2018). Thus, in order to predict the mechanism of action of this aromatic plant, we selected Eugenol (Fig. 13), the majority compound in the essential oil of species of the genus *Uvariodendron* (Parmena *et al*. 2012,Noudogbessi *et al*.2014). Prediction of its pharmacokinetic profile shows that its gastrointestinal absorption is high; it can cross the haemato-meningeal barrier and inhibits cytochrome CYP1A2, although it is not a substrate for the P-gp protein. Its skin penetration coefficient is of the order of -5.69 cm s^{-1}.

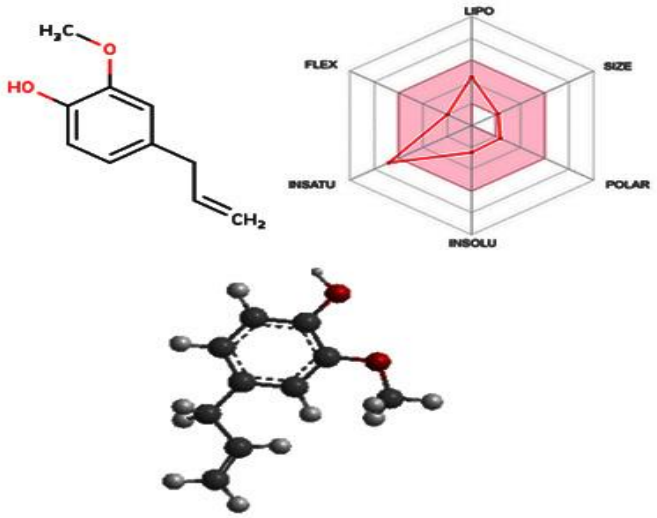

Figure 13. 2D and 3D structures of Eugenol and its physicochemical properties

Study of the interaction of this compound/ligand (Eugenol) with three receptors: haemoglobin S(ΔG°= -5.91±0.21 kcal),NF-kB (ΔG = -4.98±0.22 kcal) and Myeloperoxidase (ΔG = -5.85±0.29 kcal) indicates the formation of a thermodynamically stable complex (ΔG <0).

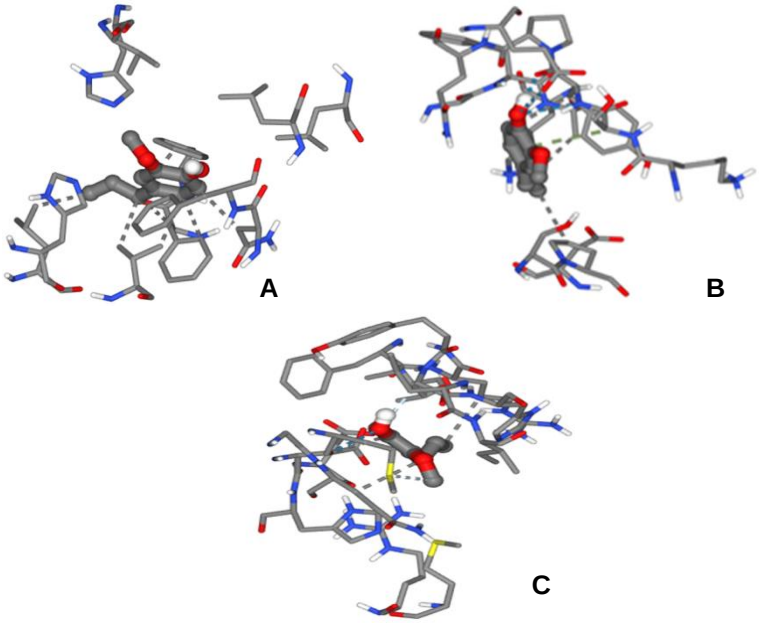

Figure 14. Complex of Eugenol with Haemoglobin S (PDB ID: 2HBS), Nuclear Factor NF-kB (3GUT) and Myeloperoxidase (1DNU)

The results of molecular docking indicate that Eugenol forms two hydrogen bonds with haemoglobin S [F103(F)O; G107(F)CA], three hydrogen bonds with nuclear factor NF-kB [E346(F)0E2; Y379(F)O; Y379(F)N] and a single hydrogen bond with myeloperoxidase [M(A)O]. Based on molecular modelling, we can therefore hypothesise that this medicinal plant acts by inhibiting haemoglobin S, nuclear factor NF-kB and myeloperoxidase respectively.

The Democratic Republic of Congo (DRC) is a reservoir of biodiversity (Asimonyio *et al*. 2015 a,b, Kambale *et al*. 2016 a,b,c, Omatoko *et al.* 2015). According to the World Health Organisation, more than 80% of the population in Africa in general, and in the DRC in particular, use NTFPs with medicinal properties to solve primary health problems (WHO 2002). The renewed interest in medicinal plants for disease management is not only a choice, but also a consequence of poverty and the high cost of modern medicines (Ngbolua *et al*.2011a,b). For this reason, there is an urgent need to assess the bio-therapeutic activity of these plant genetic resources with high biopharmaceutical potential to add value to them through the creation of factories for the production and marketing of medicines derived from traditional knowledge (Ngbolua 2014). Adding value to plants enables researchers to help the holders of traditional knowledge to improve their living conditions following the Nagoya Protocol on Access to Genetic Resources and Fair and Equitable Sharing of the Benefits Arising out of their Exploitation (Ngbolua *et al*.2016). Thus, *Uvariodendron molundense* is a NTFP with enormous potential economic value whose transformation into real income and its sustainable exploitation can contribute to human well-being.

CONCLUSION AND SUGGESTIONS/APPLICATIONS

The present study aimed to conduct a phytochemical study and evaluate the in vitro anti-sickle cell, anti-inflammatory, anti-free radical and cytotoxic activities of *Uvariodendron molundense* bark.

This study shows that:

- This plant has anti-sickle cell, anti-inflammatory and anti-free radical activities;
- Its bark powder contains fibre fragments, epidermal cell fragments, hexagonal isodiametric epidermal cells, hexagonal epidermal cells, polyhedral cells and crystalline fibre fragments;
- It contains total polyphenols, tannins, flavonoids, phenolic acids, iridoids, anthocyanins, saponosides, terpenes and coumarins;
- It is not toxic to erythrocytes.

It is therefore desirable for bioactive compounds to be isolated and characterised to formulate a antisickling phytomedicine.

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