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# Ethnobotanical and comparative study of the antioxidant and anti-inflammatory potential of three organs of *Zanthoxylum zanthoxyloides*, a plant used in the traditional treatment of sickle-cell disease in Bamako

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## Abstract

The literature reports that the sickle cell patients frequently use plants to manage their recurrent attacks. The aim of the present study was to contribute to a better understanding of the medicinal plants used in the management of that disease. An ethnobotanical survey was conducted in and around Bamako. The most frequently cited species was selected: *Zanthoxylum zanthoxyloides* Lam. Zepernick. & Timler, and the *in vitro* antioxidant and anti-inflammatory potential of their leaf, fruit and root extracts were assessed. A total of 39 people were surveyed, including 21 herbalists, 6 traditional health practitioners and 12 sickle-cell patients. Analysis of the survey forms revealed 19 plant species belonging to 16 families, including *Z. zanthoxyloides*, which had the highest frequency of citation (20.51%). The phytochemical screening of this species revealed the presence of alkaloids, phenolic compounds (flavonoids, tannins, coumarins), saponins and terpenes. The hydroethanolic extracts of *Z. zanthoxyloides* fruits showed the best DPPH free radical scavenging activity with an inhibitory concentration ($IC_{50}$) of 20.80±0.11 µg/mL followed by hydroethanolic extracts of bark with $IC_{50}$=22.9±0.14 µg/mL. In the phosphomolybdate test, the aqueous fruit extracts with a value of 537.70±0.05 mg AAE/100g exhibited the highest antioxidant potential. At a concentration of 1 mg/mL extracts, the aqueous fruit extracts demonstrated the most effective protein denaturation rates (92.66±0.66%) followed by the ethanolic root extracts (92.95±1.10%). This study showed that the fruits exhibit the highest promising anti-free radical and anti-inflammatory potential.

**Keywords:** Sickle cell disease; *Zanthoxylum zanthoxyloïdes;* Antioxidant; Anti-inflammatory

## 1. Introduction

The sickle cell disease (SCD) stands as a global health challenge, affecting millions of people around the world. It is a genetic disorder that not only causes significant suffering to those afflicted but also places a considerable burden on healthcare systems and societies at large. SCD is characterized by the abnormal shape of red blood cells, which can lead to a multitude of health complications and a reduced quality of life for those affected.

Today, this pathology has become an international problem due to migratory flows and the brewing of genes within populations [1]. It is the most common genetic disorder in the world, with approximately 500,000 new cases annually, with roughly half of these occurrences recorded in the African continent alone. In Mali, the statistics showed an average sickle cell gene prevalence of 12% and an annual number of sickle cell births between 5000 and 6000 [2]. The SCD inflicts a range of debilitating crises on those it afflicts. These include respiratory crises, triggered by altered oxygen

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transport; inflammatory crises, resulting from the release of inflammatory proteins; and oxidative crises, induced by an excess of reactive oxygen species. Together, these crises significantly impact the health and well-being of individuals living with SCD [3][4]. In addition to these crises, patients see themselves as "burdens" for their families. With their low incomes, these families faced a daily dilemma i.e. choosing between the expense of caring for a sickle cell child and the survival and education needs of other children [2].

Various therapeutic options have been proposed to combat sickle cell disease, in order to lighten the suffering of patients and their families. However, it has to be recognized that all these therapeutic approaches have side effects and are often inaccessible to low-income populations. That's why, progress has been made through phytotherapeutic approaches. The *in vitro* antifalcemic activity of several plant species used in traditional African medicine against sickle cell disease has been validated [1][5][6] and a few active molecules have been isolated [7][8]. Several anti-sickle cell improved traditional medicines have achieved this success: ''VK500'' in Benin, ''Faca'' in Burkina Faso and ''Fagara'' in Mali. However, the majority of these phytomedicines are derived from the bark and roots of a highly coveted species: *Zanthoxylum zanthoxyloides* Lam. Zepern. & Timler. Without gene therapy, these bark- and root-based phytomedicines enhance the management of oxidative and inflammatory crises due to their abundant antioxidant and anti-inflammatory compounds [5][9].

Nevertheless, the use of these two organs has harmful effects, such as blocking sap transport [10], increasing the risk of insect attack [11] and could lead to the disappearance of the species. Regardful about the threat to the *Z. zanthoxyloides* species, this study was initiated to conduct a comparative study of the *in vitro* antioxidant and anti-inflammatory potential of extracts from its organs, including fruit.

## 2. Material and methods

### 2.1. Sites of survey

The survey was carried out at five sites in Bamako and Kati (Figure 1). Bamako was represented by the Commune III, Commune V, Commune VI and Kati was represented by Banankoro.

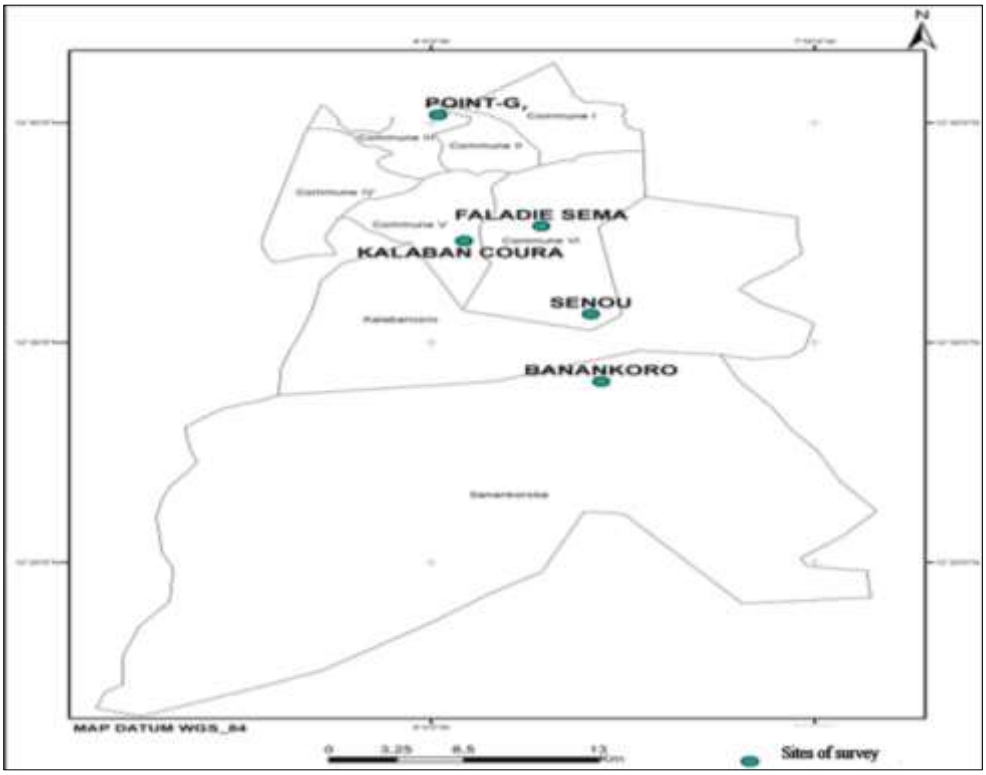


**Figure 1** Survey sites

## 2.2. Plant material

The plant material consisted of fruits, roots and trunk bark of *Z. zanthoxyloides*, harvested in Banankoro. Samples were carefully washed, dried at room temperature, powdered and then stored in a dark and dry place.

## 2.3. Methods

### *2.3.1. Ethnobotanical survey*

The ethnobotanical survey took place from July to October 2021. Data were collected using a questionnaire form and individual interviews. The local language "Bamanakan" was used for the interviews. The targeted individuals who had given their verbal consent were interviewed.

The questionnaire considered the socio-demographic characteristics of the respondents (age, sex, ethnic group, etc.), the local names of the plants used to treat sickle cell disease, the parts used, and their preparation and administration methods. Botanical and Ecotoxicology Laboratory of University of Sciences, Techniques and Technologies of Bamako (USTTB) identified the plant species mentioned.

### *2.3.2. Experimental study*

Preparation of extracts

The extracts were obtained by maceration of 10% (w/v) powder of each organ in solvent (distilled water or 70% ethanol). The mixture was placed under magnetic stirring at room temperature for 24 h, then filtered under vacuum. The residue obtained was subjected to the same operation again. The recovered filtrates were concentrated using an evaporator and stored in a cool place for subsequent analysis.

Phytochemical composition

The phytochemical screening of extracts was carried out using qualitative characterization techniques, according to standard methods described by Harbone [12] and Bruneton [13]. The total phenolic compounds and flavonoid contents were estimated by spectrophotometry, using respectively Folin Ciocalteu and aluminum chloride tests reported by Konaré et al. [14]. The results were expressed in milligrams acid ascorbic equivalents per gram of dry matter (mg AAE/g DM) for phenolic compounds and milligrams quercetin equivalents per gram of dry matter (mg QE/g DM) for flavonoids.

Antioxidant activity

- Total antioxidant capacity (TAC) or Phosphomolybdate test (PPM)

The total antioxidant capacity (TAC) of plant extracts was assessed by the phosphomolybdate method used by Konaré et al. [14]. A volume of 0.1 mL of each extract was mixed with 0.9 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Tubes are incubated at 90°C for 90 min. After cooling, the absorbances of the solutions were read at 695 nm against a distilled water blank. The calibration line is based on a standard quercetin solution treated under the same conditions as the extracts. Total antioxidant capacity was expressed in equivalent milligrams of ascorbic acid per 100 grams of dry matter (mg AAE/100g DM).

- DPPH test

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) test was used according to the protocol described by Brand-Williams [15], slightly modified by Togola et al. [16]. Briefly, 50 µL of each ethanolic solution at different concentrations is added to 1.95 mL of DPPH ethanolic solution (0.024 g/L). At the same time, a negative control was prepared by mixing 50 µL of ethanol with 1.95 mL of DPPH ethanolic solution. Absorbance readings were taken using a spectrophotometer against a blank at 515 nm after 30 min incubation in the dark at room temperature. The positive control is represented by a solution of a standard antioxidant, ascorbic acid. The free radical scavenging activity of the DPPH radical was expressed in inhibition rate calculated from the absorbances obtained according to the following formula:

$$\text{Inhibition rate (\%)} = [1- (\text{Sample Absorbance} / \text{Blank Absorbance})] \times 100 \quad (1)$$

- Anti-inflammatory activity: Protein denaturation test

The anti-inflammatory activity of the extracts was estimated based on the bovine serum albumin (BSA) denaturation assay according to the protocol reported by Fetni and Bertella [17]. The reaction mixture consisted of 1 mL bovine serum albumin (BSA) solution, 3 mL phosphate-buffered saline (PBS, pH 6.4) and 1 mL of variable extract concentrations (0 to 1000 µg/mL). A similar volume of distilled water served as control (blank). The mixtures were incubated at 37° C for 15 min, then heated to 70°C for 5 min. After cooling, the absorbances were read at 660 nm. Diclofenac sodium was used as the reference molecule. The negative control represents 100% protein denaturation. The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ Inhibition} = \left(1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of negative control}}\right) \times 100 \quad (2)$$

## 3. Results

### 3.1. Ethnobotanical survey

*3.1.1. Socio-demographic characteristics of respondents*

The main data collected in the Communes of Bamako and the Cercle of Kati are the socio-demographic characteristics of the people surveyed. The Table 1 shows the distribution of respondents by gender, district and status.

**Table 1** Distribution of respondents by gender, location and status

| **Variables** | **Sites** | **Frequencies (%)** | | |
|---|---|---|---|---|
| | | **Male** | **Female** | **Total** |
| Zones of survey | Point G | 10.26 | 20.51 | 30.77 |
| | Faladie Sema | 5.13 | 15.38 | 20.51 |
| | Kalaban Coura | 2.56 | 10.26 | 12.82 |
| | Senou | 7.69 | 12.82 | 20.51 |
| | Banankoro | 5.13 | 10.26 | 15.38 |
| | Total | 30.77 | 69.23 | 100.00 |
| Status of respondents | Sickle cell patients | 10.26 | 20.51 | 30.77 |
| | Traditional healers | 10.26 | 5.13 | 15.38 |
| | Sellers of medicinal plant | 10.26 | 43.59 | 53.85 |
| | Total | 30.77 | 69.23 | 100.00 |

A total of 39 people were surveyed, with 12 from Point G (Commune III), 5 from Kalaban Coura (Commune IV), 8 from Faladie Sema and Senou (Commune VI) and 6 from Banankoro (Kati). Among these interviewed people, 12 were sickle cell patients (30.77%), 6 traditional healers (15.38%) and 21 local plant sellers (53.85%). The Table 1 also indicated a predominance of women in the survey (69.23%).

*3.1.2. Medicinal plants recorded*

The Figure 2 shows a list of different species identified during the ethnobotanical survey, and their number of citations.

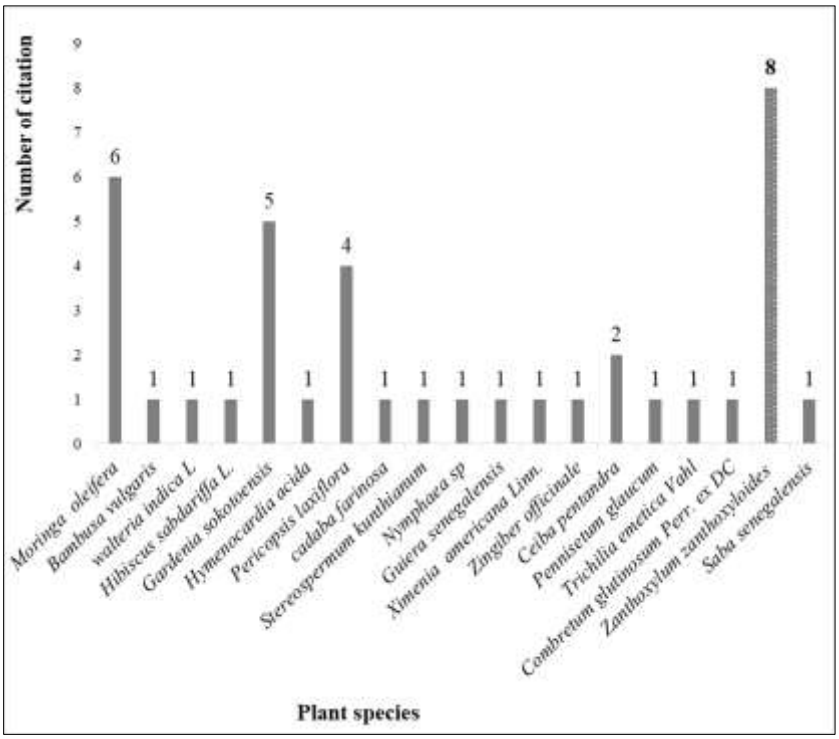


**Figure 2** Listed species and their number of citations

The survey allowed to record 19 species belonging to 16 families traditionally used to treat sickle cell crises. The most cited species was *Zanthoxylum zanthoxyloïdes* with 20.51% of citation (Figure 2), hence the choice of this species for biological activities.

*3.1.3. Plant distribution according to botanical families*

The different plant families identified during the survey are shown in Figure 3. The Combretaceae, Malvaceae and Poaceae families are the most represented, with 2 species each.

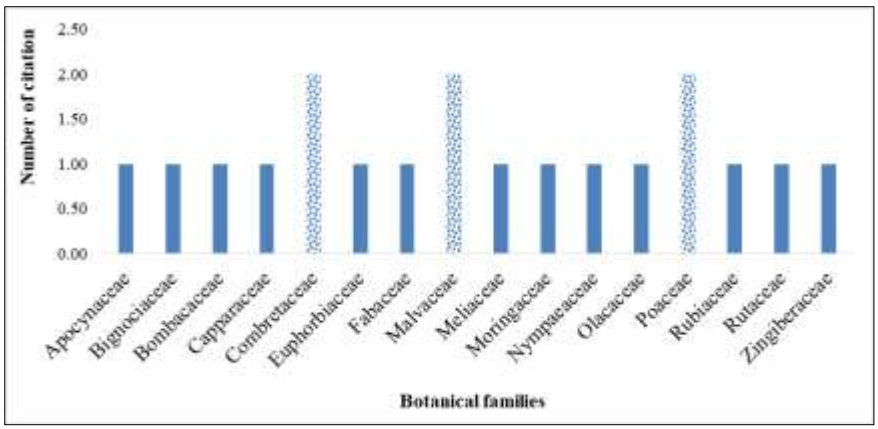


**Figure 3** Distribution of species by botanical family

*3.1.4. Plant organs used*

The different organs used in the traditional treatment of sickle-cell anemia are summarized in Figure 4.

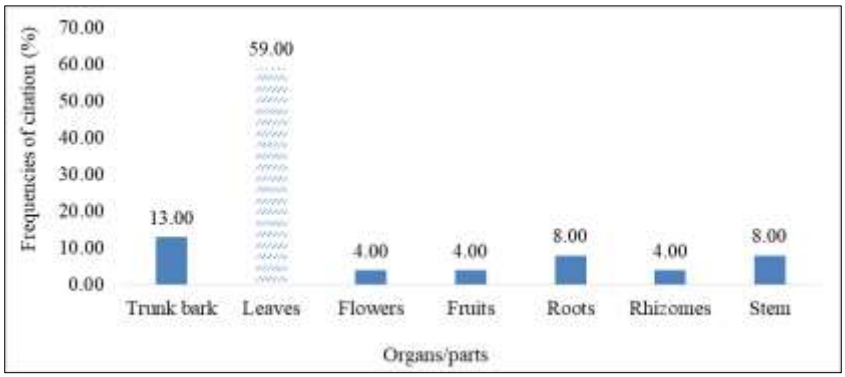


**Figure 4** Frequencies of used organs

Among the various plant parts used by sickle-cell patients, the leaves (59%) were the most used, followed by trunk bark (13%), roots and stems.

*3.1.5. Preparation methods and routes of administration*

The Figures 5 and 6 illustrate the different preparation methods and routes of administration for the listed species.

The analysis of Figure 5 shows that decoction is the most popular preparation method with 75%, followed by infusion and maceration/granulation. In this survey, the most common route of administration was oral (53%), followed by bathing, fumigation and use of cataplasm (Figure 6).

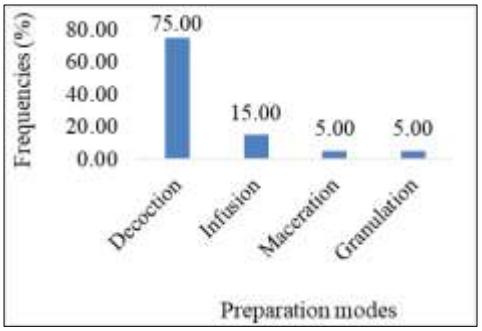


**Figure 5** Modes of preparation

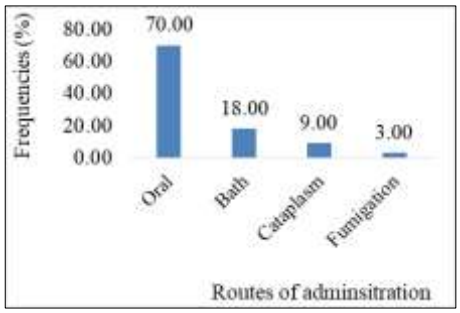


**Figure 6** Modes of administration

### 3.2. Phytochemical composition

**Table 2** Phytochemical composition

| Chemical Groups | Organs and extracts | | | | | |
|---|---|---|---|---|---|---|
| | Fruits | | Trunk bark | | Roots | |
| | Aqueous | hydroethanolic | Aqueous | hydroethanolic | Aqueous | hydroethanolic |
| Alkaloids | + | + | + | + | + | + |
| Tannins | + | + | + | + | + | + |
| Flavonoids | + | + | + | + | + | + |
| Saponines | - | - | - | - | + | + |
| Coumarins | + | + | + | + | + | + |
| Terpenes | + | + | + | + | + | + |

*Presence: (+) Absence: (-)

The results of phytochemical screening of the various extracts from the three organs of *Z. zanthoxyloides* are presented in Table 2. The results for total phenolic compounds and flavonoids are shown in Table 2.

The Table 2 shows the presence of alkaloids, tannins, coumarins, flavonoids and terpenes in all extracts. In contrast, saponins were exclusively detected in root extracts.

The data in Table 3 show that the aqueous extract of trunk bark contained the highest amount of total phenolic compounds (234.00 ± 0.001 mg EAG/g), and the lowest was recorded in the hydroethanolic extract of roots (99.00 ± 0.009 mg GAE/g). As for flavonoids, the aqueous fruit extract showed the highest level with 12.60 ± 0.017 mg QE/g against 3.30 ± 0.006 mg QE/g for the aqueous root extract.

**Table 3** Total phenolic compounds and flavonoid content of extracts

| Organs | Extracts | Phenolic compounds (mg GAE/g) | Flavonoids (mg QE/g) |
|---|---|---|---|
| Fruits | Aqueous | 192,00 ± 0,010$^{b}$ | 12,6 ± 0,017$^{a}$ |
| | Hydroethanolic | 158,00 ±0,005$^{c}$ | 11,80 ± 0,034$^{a}$ |
| Trunk barks | Aqueous | 234,00 ± 0,001$^{a}$ | 5,60 ± 0,010$^{c}$ |
| | Hydroethanolic | 178,00 ± 0,009$^{b}$ | 3,40 ± 0,007$^{d}$ |
| Roots | Aqueous | 152,00 ± 0,010$^{c}$ | 3,30 ± 0,006$^{d}$ |
| | Hydroethanolic | 99,00 ± 0,009$^{d}$ | 9,4 ± 0,022$^{b}$ |

*Different letters (a-d) for the same parameter show a significant difference between means at 0.05.

### 3.3. *In vitro* antioxidant potential

#### *3.3.1. Total antioxidant capacity (TAC)*

The Figure 7 shows the results for total antioxidant capacity.

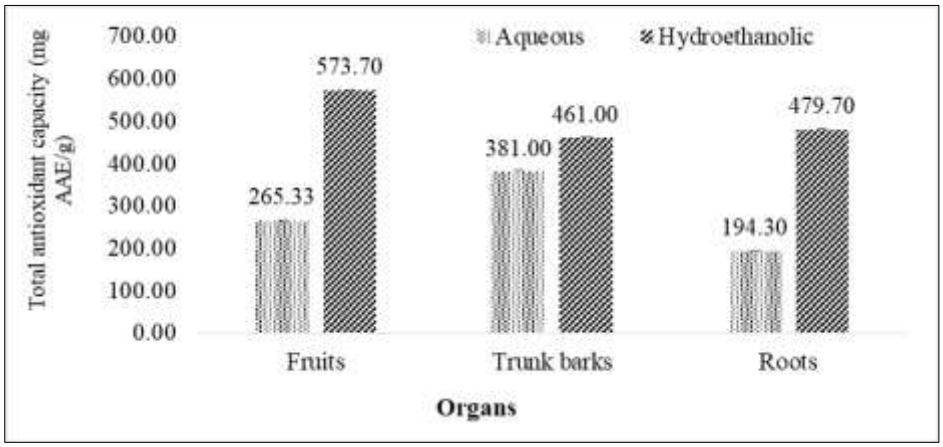


**Figure 7** Diagrams expressing total antioxidant capacity of extracts

This figure reveals that TAC varied according to extracts and organs. The highest activity was registered in the fruit hydroethanolic extract with 537.7 ± 0.05 mg AAE/100g DM, and the lowest in the root aqueous extract with 194.3 ± 0.05 mg AAE/100g DM.

#### *3.3.2. DPPH test*

The results obtained with the DPPH Test are translated into 50% inhibitory concentrations ($IC_{50}$ expressed in µg/mL) (Table 2).

**Table 4** Anti-radical activity expressed as $IC_{50}$

| Organs | Extracts | $IC_{50}$ (µg/mL) |
|---|---|---|
| Fruits | Aqueous | 23.70±0.11$^{c}$ |
| | Hydroethanolic | 20.83±0.11$^{d}$ |
| Trunk barks | Aqueous | 22.93±0.14$^{c}$ |
| | Hydroethanolic | 25.80±0.56$^{a}$ |
| Roots | Aqueous | 24.97±0.25$^{b}$ |
| | Hydroethanolic | 24.72±0.15$^{b}$ |

*For each extract, means do not share any letter are significantly different at the 0.05 threshold.

The results of the DPPH test showed that the ethanolic fruit extracts with the lowest $IC_{50}$ (20.80±0.11 µg/mL) were the richest in antioxidants.

### 3.4. Anti-inflammatory activity: effect of protein denaturation

The inhibitory effects of extracts from different organs at different concentrations on protein denaturation are shown in Table 5. This table reveals that the aqueous and ethanolic extracts of the different organs of *Z. zanthoxyloides* exhibited good inhibition of protein denaturation, with variation in the inhibition rate from one extract to another and from one organ to another (p-value < 0.05). The aqueous fruit extract and hydroethanolic root extract at 1000 µg/mL showed the highest inhibition rates of 92.66±0.66% and 92.95±1.10%. These results are statistically similar to those observed with Diclofenac sodium (93.33±1.53%), the positive control.

**Table 5** Inhibitory effects of protein denaturation of extracts

| Extracts | Organs | Concentrations (µg/mL) | | | | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | 31,25 | 62,5 | 125 | 250 | 500 | 1000 | |
| Aqueous | Fruits | 70.92±0.44$^{Ec}$ | 72.27±0.44$^{Ee}$ | 77.78±0.7$^{Dc}$ | 80.77±0.60$^{Cd}$ | 89.95±1.93$^{Bb}$ | 92.66±0.66$^{Aa}$ | 0.001E-8 |
| | Barks | 73.04±0.77$^{Ebc}$ | 75.65±1.33$^{Dcd}$ | 78.55±1.33$^{Cc}$ | 81.84±0.73$^{Bcd}$ | 84.25±0.73$^{Bd}$ | 85.12±0.44$^{Ac}$ | 0.001E-5 |
| | Roots | 73.04±1.16$^{Fbc}$ | 74.98±0.44$^{Ede}$ | 78.16±1.60$^{Dc}$ | 82.71±0.44$^{Cc}$ | 85.12±0.44$^{Bcd}$ | 86.86±1.37$^{Ab}$ | 0.001E-5 |
| Hydro-ethanolic | Fruits | 73.04±1.16$^{Cbc}$ | 79.71±1.05$^{Bb}$ | 83.38±1.60$^{Ab}$ | 84.73±0.60$^{Ab}$ | 84.73±0.60$^{Acd}$ | 84.54±0.67$^{Acd}$ | 0.003E-5 |
| | Barks | 75.75±1.70$^{Bab}$ | 77.97±3.05$^{Bbc}$ | 81.93±1.70$^{Ab}$ | 82.90±0.29$^{Ac}$ | 83.38±0.58$^{Ad}$ | 83.19±0.58$^{Ad}$ | 0.0002 |
| | Roots | 78.07±3.13$^{Da}$ | 84.25±1.43$^{Ca}$ | 89.37±0.33$^{Ba}$ | 90.72±0.50$^{ABa}$ | 91.79±0.44$^{ABa}$ | 92.95±1.10$^{Aa}$ | 0.003E-4 |
| positive control | Diclofenac | 44.35±3.05$^{Ed}$ | 62.22±1.77$^{Df}$ | 74.98±2.53$^{Cd}$ | 78.36±1.02$^{Ce}$ | 88.21±1.89$^{Bc}$ | 93.33±1.53$^{Aa}$ | 0.002E-8 |
| P-value | | 0.001E-7 | 0.001E-5 | 0.005E-4 | 0.001E-7 | 0.001E-3 | 0.003E-6 | |

*For each extract, the means sharing no capital letters are significantly different. For each concentration, the means sharing no lower-case letters are significantly different.

## 4. Discussion

### 4.1. Ethnobotanical survey

The results of the ethnobotanical survey revealed that sickle cell patients living in and around Bamako use 19 plant species belonging to 16 families for the management of sickle cell crises. The most cited species was *Z. zanthoxyloïdes*,

called "Wo" in the local Bamanakan language, with 20.51% as frequency of citation. The leaves were the most frequently used organs (59%), followed by the barks (13%) and the roots (8%). These results are in agreement with the work of Dembélé [6], who during a survey of sickle cell patients members of AMLUD (Malian Association for the Fight against Sickle Cell Disease) in Bamako, showed that the *Z. zanthoxyloïdes* species was widely used and that the leaves were the most cited organs in the treatment of sickle cell disease. Mpiana et al. [1] have also reported the very frequent use of *Z. zanthoxyloides* leaves in the management of sickle cell disease. On the other hand, the literature reports an abusive use of the barks and roots of this species in the design of numerous phytomedicines in Africa [5][6][9]. This interest in leaves by sickle-cell patients could be linked to their richness in bioactive compounds. This use should be encouraged insofar as it is very important not only for the survival of the species but also the leaves are easily and abundantly renewed [18].

### 4.2. Phytochemical composition

The phytochemical screening of the three organs studied revealed the presence of several secondary metabolites such as alkaloids, phenolic compounds (flavonoids, tannins, coumarins) and terpenes. In opposite to other compounds, saponins were found only in root extracts. The presence of these metabolites had already been reported in some previous works of Olushola-siedoks et al. [19] and Cisse et al. [9]. However, Dembélé [6] has reported the presence of saponins in root and bark extracts and the absence of flavonoids in roots and trunk bark of the same species. The literature reports that intraspecific differences may be multifactorial in origin (geographical origin of the species, harvesting period, maturity stage, etc.).

These data reveal that the organs of *Z. zanthoxyloides* are very rich in secondary metabolites, and this pharmacological richness could justify their use in the traditional treatment of sickle cell disease. The work of Cisse et al. [9] reported that the fruits, barks and leaves of this species were used in the treatment of anemia, thanks to their fairly high iron content (1 to 7 mg/L). This could justify the use of these organs in sickle cell patients who regularly suffer from anemia attacks.

All these compounds are known for their physiological activities and medicinal properties. For instance, the alkaloids, tannins and flavonoids have been shown to promote tissue regeneration, reduce the permeability of blood capillaries and enhance their resistance to hemolysis [20][21]. These molecules are involved in the management of several diseases such as sickle cell disease [22].

The spectrophotometric assay showed that aqueous extracts of bark contained the highest levels of phenolic compounds (234.00 ± 0.001 mg GAE/g), followed by fruit (192.00 ± 0.010 mg GAE/g), and the lowest levels were recorded in hydroethanolic extracts of roots (99.00 ± 0.009 mg GAE/g). These values are higher than those obtained by Phuyal et al. [23] with methanolic extracts, which were 185.15 ± 1.22 mg GAE/g for barks and 185.15 ± 1.22 mg GAE/g for fruits of *Zanthoxylum armatum* species. In contrast, the levels reported by Ouedraogo et al. [24] with methanolic extracts of *Z. zanthoxyloides* bark (7.85 ± 0.32 mg GAE/100mg) are lower than ours.

As for flavonoids, the aqueous extracts showed the highest levels (p-value < 0.05), with 12.6 ± 0.017 mg QE/g for fruits against 3.30 ± 0.006 mg QE/g for roots. These data are good news insofar as the aqueous form, which is not or is less toxic, remains the main form of medicinal plant use [25][26]. Ouedraogo et al. [24] reported less interesting flavonoid contents with ethanolic bark extracts (1.12 ± 0.01 mg QE/100 mg). On the other hand, flavonoid contents reported by Tine et al. [27] with methanolic extracts of fruits of the same species (49.61 mg QE/mg) are higher than ours.

The phenolic compounds, particularly the flavonoids, are known for their antioxidant, anti-inflammatory, anticancer and antitumor properties [28][29]. With these significant levels of flavonoid, the fruits could potentially replace barks and roots in traditional use, reducing the pressure on plant species at risk of disappearing. The work of Ouattara et al. [7] in Burkina Faso aroused a new interest in roots, with the isolation of three new bioactive compounds (known as Burkinabins A, B and C) from root bark extracts. While these compounds were able to reduce the number of sickle cells from 11.4% to 67.3%, studies have shown that these anti-sickle cell properties are due to the involvement of other chemical groups such as phenolic compounds. The phenolic compounds, and singularly the flavonoids, are known for their antioxidant, anti-inflammatory, anticancer, antitumor and antisickling properties [28][30]. Based on these amounts of flavonoid, the fruits of *Z. zanthoxyloides* could be suggested as an alternative to replace barks and roots, whose current use would undoubtedly lead to the species' extinction.

### 4.3. Antioxidant et anti-inflammatory potential

The respiratory and inflammatory crises are very frequent in sickle cell patients as a result of excessive production of active oxygen derivatives (AODs) [3] and denatured proteins [4] respectively. Consequently, the management of these

crises involves those of these AODs and denatured proteins through anti-inflammatory and antiradical agents contained in plant extracts.

The evaluation of free radical scavenging capacity showed the highest activity in the hydroethanolic fruit extracts with 537.7 ± 0.05 mg AAE/100g DM for the phosphomolybdenum test, and the lowest in the aqueous root extract with 194.3 ± 0.05 mg AAE/100g DM. The same trends were confirmed by the results of DPPH test, which showed that the hydorethanolic fruit extracts had the lowest $IC_{50}$ (20.80±0.11 µg/mL), and therefore the highest antioxidant content. Our results are higher than those of Tanoh [31], who obtained an $IC_{50}$ of 58.10 ± 1.20 µg/mL with *Z. mezoneurispinosum* trunk bark, and also with *Z. psammophilum* roots (45.80 ± 0.10 µg/mL) and *Z. leperieurii* fruits (103.55±0.35) µg/mL). Similarly Ayoka et al. [32] have reported low levels in *Z. zanthoxyloides* leaves (94.41±0.18 µg/mL). In their studies on isolated mitochondria Adekunle et al. [33] had demonstrated the ability of *Z. zanthoxyloides* extracts to scavenge free radicals, thus reducing the production of reactive oxygen species inside these mitochondria.

Known for their antioxidant activity, the presence of the phenolic compounds, especially flavonoids, is believed to play a crucial role in the antioxidant properties of plants used to treat sickle cell disease in traditional medicine. These properties would stabilize the membrane of SS erythrocytes and reduce the $Fe^{3+}/Fe^{2+}$ ratio [1][21]. Likewise, other studies have shown that phenolic compounds, due to their acylation capacity, possess the ability to reduce the number of sickle cells (S red blood cells) from 75% to 30%. This reduction would increase the affinity of hemoglobin for oxygen [8]. Thus, the investigated extracts, with their significant levels in these compounds, could contribute to a better management of respiratory crises in sickle cell patients.

During the inflammation, many of the involved proteins are denatured. This is why the protein anti-denaturation assay is used to estimate the anti-inflammatory activity of plant extracts. The aqueous and hydroethanolic extracts of *Z. zanthoxyloides* fruits, barks and roots showed good inhibition of protein denaturation, ranging from 70.92±0.44% to 92.95±1.10%. These inhibitory effects were extract and organ-dependent (p-value < 0.05). At 1000 µg/mL, aqueous fruit extract with 92.66±0.66% and hydroethanolic root extract with 92.95±1.10% showed the best inhibition rates, statistically similar to that observed with Diclofenac sodium (93.33±1.53%). These data corroborate those reported by Thejashwini et al. [34] with methanolic extracts of *Z. rhetsa* fruits, which had a protein inhibition rate of 85%. Numerous studies have shown that polyphenols inhibit the enzymatic activities of lipid metabolism and reduce the production of inflammatory mediators such as nitrogen monoxides, prostaglandins and leukotrienes [35][30]. The polyphenols are therefore responsible for anti-inflammatory activities, hence their use as chemopreventive agents.

The anti-inflammatory effect of sterols, terpenes and saponins has also been reported and may be due to an inhibition of cyclooxygenase and the release of pro-inflammatory cytokines [22]. The fruits with their high content in phenolic compounds could be a potential candidate to replace validly the barks and roots. The work of Diatta et al. [36] had already suggested the use of *Z. zanthoxyloides* leaves in place of roots, in pathologies such as sickle cell disease, in view of their efficacy on inflammation and pain. These data support the conclusions formulated by Togola et al. [16] to replace the bark of *Anarcadium occidentale*, widely used against hypertension and diabetes in Mali, with its leaves. This hypothesis was also based on the wealth of phenolic leaves and antioxidant agents.

## 5. Conclusion

The data from this study showed that several plant species are used by local populations in Bamako in the management of sickle cell disease. Among these plant species used, *Z. zanthoxyloides* was the most coveted. The phytochemical investigations carried out on this species highlighted its richness in bioactive compounds. The leaf, fruit and root extracts were also found to have interesting antioxidant and anti-inflammatory potential. The hydroethanolic and aqueous fruit extracts showed the best free radical scavenging properties and protein denaturation inhibition rates at 1000 µg/mL. Based on these interesting activities recorded with the fruits, they could be suggested to replace the barks and roots in the formulation of phytomedicines for a better safeguarding of the species. To validate this hypothesis, it would be necessary to carry out further tests evaluating the antisickling and antihemolytic activities of fruit extracts.

## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interest in the present work. All authors read and approved the final version of the manuscript.

### *Statement of ethical approval*

The present research work does not contain any studies performed on animals/humans subjects by any of the authors.

### *Statement of informed consent*

Informed consent was obtained from all individual participants included in the study.

## References

[1] Mpiana PT, Ngbolua K-N, Tshibangu STD. Les alicaments et la drépanocytose : une mini-revue. Comptes rendus Chim. 2016;19:884–9.

[2] Guindo A, Sarro YS, Touré BA, Keita I, Baraika MA, Coulibaly M, et al. Le Centre de recherche et de lutte contre la drépanocytose de Bamako : histoire, bilan, défis et perspectives. Press Med Form. 2021;10:8.

[3] Nur E, Biemond BJ, Otten HM, Brandjes DP, Schnog JJB. Oxidative stress in sickle cell disease; pathophysiology and potential implications for disease management. Am J Hematol. 2011;86(6):484–9.

[4] Dahmani F, Benkirane S, Kouzih J, Woumki A, Mamad H, Masrar1 A. Etude de l'hémogramme dans la drépanocytose homozygote: à propos de 87 patients. Pan Afr Med J. 2016;8688:1–10.

[5] Ouédraogo JCW, Kini FB, Belemnaba L, Ouédraogo S, Guissou PI. Standardisation de la poudre des écorces de racine de Zanthoxylum zanthoxyloides en vue la production d'un phytomédicament anti- drépanocytaire pédiatrique. J la Société Ouest-Africaine Chim. 2015;040:24–30.

[6] Dembélé. Etude des plantes medicinales utilisées dans la prise en charge de la drépanocytose à Bamako (Mali). Thèse de Pharmacie, 128p., Faculté de Pharmacie, Université des Sciences, des Techniques et des Technologies de Bamako, Bamako, Mali; 2018.

[7] Ouattara B, Jansen O, Angenot L, Guissou I, Frédérich M, Fondu P, et al. Antisickling properties of divanilloyl_quinic acids isolated from Fagara zanthoxyloides Lam. (Rutaceae). Phytomedicine. 2009;16:125–9.

[8] Ngbolua K, Mpiana PT, Tshibangu DS-T, Gbolo BZ. Bioactivity of medicinal plants traditionally used for the management of sickle cell disease in Democratic Republic of the Congo : State of the art and future directions. Marilyn E Lewis (ed), Sick Cell Dis Genet Manag Progn Nov Publ New York, USA. 2015;Chapter 4:79–94.

[9] Cissé S, Somboro AA, Cissé M, Bouaré S, Tangara O, Dembélé N, et al. Contribution to the ethnobotanical and phytochemical study of Zanthoxylum zanthoxyloides (Lam.) Waterman (Rutaceae) from Mali. J Drug Deliv Ther. 2022;12(6):10–5.

[10] Delvaux C, Sinsin B, Darchambeau F, Van Damme P. Recovery from bark harvesting of 12 medicinal tree species in Benin, West Africa. J Appl Ecol. 2009;46(703–712).

[11] Mbinile SD, Munishi LK, Ngondya IB, Ndakidem PA. Conservation and Management Challenges Facinga Medicinal Plant Zanthoxylum chalybeum in Simanjiro Area, Northern Tanzania. Sustainability. 2020;12:4140.

[12] Harbone JB. Phytochimique Methods: A guide to moderne techniques of plant analysis. 3ème Editi. chapman and Hall; 1998. 303 p.

[13] Bruneton J. Pharmacognosie, phytochimie, plantes médicinales. 4ème Editi. 2009;1268.

[14] Konaré MA, Diarra N, Cissé C, Traoré DAK, Togola I, Kassogué A, et al. Evaluation of the biological activities of leaf and bark extracts of Ficus platiphylla Delile , a medicinal plant used in Mali. J Med Plants Res. 2020;14(3):118–28.

[15] Brand-Williams W, Cuvelier M, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci ence Technol. 1995;28(1):25–30.

[16] Togola I, Kaya Y, Diarra N, Konare MA, Denou A, Sanogo R. Comparative Study of the Phytochemistry and Antioxidant Activity of Anacardium occidentale (L.) Leaf and Stem Bark Extracts. J Dis Med Plants. 2020;6(3):72–6.

[17] Fetni S, Bertella N. Etude in vitro des propriétés anti-inflammatoires de l'extrait méthanolique des fruits de Rosa canina L. (Rosaceae). Nutr Santé. 2020;09(02):117–25.

[18] Diarra ML, Mariko M, Samba M, Noba K. Plantes médicinales utilisées dans le traitement traditionnel du paludisme à Bamako ( Mali ). Int J Biol Chem Sci. 2016;10(4):1534–41.

[19] Olushola-siedoks AA, Igbo UE, Asieba G, Damola IA, Igwe CC. Elemental analysis and phytochemical characterisation of Zanthoxylum zanthoxyloides ( Lam .) Zepern . and Timler stem bark. J Pharmacogn Phytochem. 2020;9(5):41–6.

[20] Railson H, Michel FO, Aline EEF, Priscilia H, Aguinaldo JDN, Maria SSL. Effets protecteur contre des flavonoides contre la production d especes reactives de l oxygene chez les patients atteints d'anémie falciforme traités par hydroxyurée. Rev Bras Hematolgia Hemoterterapia. 2013;35:52–5.

[21] Sarode SA, Sonawane YN, Suralkar RK, Kumbhar DD, Warade PP, Patil PR. Moringa Oleifera: Phytochemistry, pharmacology. GSC Biol Pharm Sci. 2023;24(03):041–55.

[22] Koudou JK, Shcherazade F, N'guessan Z, Kouakou KR, Kouassi BE, Kplé MK, et al. Activité anti-inflammatoire et etudes phytochimiques de l'extrait aqueux des ecorces de Distemonanthus Benthamianus Baill. Eur Sci J. 2021;17(7):75–93.

[23] Phuyal N, Jha PK, Raturi PP, Rajbhandary S. Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and Bark Extracts of Zanthoxylum armatum DC. Sci World J. 2020;1–7.

[24] Ouedraogo V, Compaoré E, Rouamba A, Compaoré A, Kiendrebeogo M. Anti-virulence Activity of Three Medicinal Plants: Cassia occidentalis L., Crossopteryx febrifuga (Afzel ex G. Don) Benth. and Zanthoxylum zanthoxyloides (Lam) Zep. and Timl. Microbiol Res J Int. 2019;29(1):1–7.

[25] Traoré K, Haidara M, Denou A, Dembélé SM, Sanogo R. Plantes médicinales utilisées dans la prise en charge des maladies infantiles dans quatre zones écologiques et culturelles du Mali. Rev RAMReS – Série Pharmacopée Médiecine Tradit Africaine. 2023;22(1):91–102.

[26] Dembélé J, Togola I, Daou C, Diarra N, Konaré MA. Evaluation of the Antioxidant Activity of Some Plants Used in the Management of Erectile Dysfunction by the BWA Community in Mali. J Drug Deliv Ther. 2020;10(6):57–61.

[27] Tine Y, Yang Y, Renucci F, Costa J, Wélé A, Paolini J. LC-MS/MS Analysis of Flavonoid Compounds from Zanthoxylum zanthoxyloides Extracts and Their Antioxidant Activities. Nat Prod Commun. 2017;12:1865–8.

[28] Cowan M. Plant product as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564–82.

[29] Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. Vol. 6, Journal of Pharmaceutical Analysis. 2016.

[30] Odibo AO, Akaniro IR, Ubah EM. In vitro investigation of the antisickling properties of aqueous fruits extracts of Citrus paradisi, Musa acuminata and Malus domestica. GSC Biol Pharm Sci. 2020;13(3):203–9.

[31] Tanoh AE. Etude phytochimique et biologique de trois plantes du genre Zanthoxylum. Thèse de Doctorat, 296p.; Université Félix Houphouët Boigny – Abidjan (Côte d'ivoire) et Université de Liège - Gembloux Agro-Bio Tech (Belgique); 2021.

[32] Ayoka TO, Nwachukwun N, Ene AC, Igwe CU, Nnadi CO. Liquid chromatography-mass spectrometric analyses of potential antioxidant constituents from Zanthoxylum zanthoxyloides leaves: Probing into the role of alkaloids. Trop J Nat Prod Res. 2020;4(10):817–23.

[33] Adekunle A, Kamdem JP, Rocha JBT. Antioxidant Activity and HPLC Analysis of Zanthozylum zanthoxyloides. Rep Opin. 2012;4(3):6–13.

[34] Thejashwini BR, Shilpashree M, Kiran Kumar DJ, Rama T. Phytochemical analysis and evaluation of Zanthoxylum rhetsa ( roxb ) for it's in vitro pharmocological properties. Res J Life Sci Chem Sci. 2020;6(4):104–18.

[35] Guo W, Kong E, Meydani M. Dietary Polyphenols, Inflammation and Cancer. Nutr Cancer. 2009;61(6):807–10.

[36] Diatta W, Sy GY, Manga CI, Diatta K, Dior A, Bassene E. Recherche des activités anti-inflammatoire et analgésique des extraits de feuilles de Zanthoxylum zanthoxyloides ( Lam ) zepernick et timler ( Rutaceae ). Int J Biol Chem Sci. 2014;8(1):128–33.