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Mahamane Haidara
1) Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako (USTTB), Mali, West Africa
2) National Institute for Research on Traditional Medicine and Pharmacopoeia (NIRTMP), Mali, West Africa

Daouda L Dembele
Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako (USTTB), Mali, West Africa

Sékou Doumbia
Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako (USTTB), Mali, West Africa

Rokia Sanogo
1) Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako (USTTB), Mali, West Africa
2) National Institute for Research on Traditional Medicine and Pharmacopoeia (NIRTMP), Mali, West Africa

Corresponding Author:
Mahamane Haidara
1) Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako (USTTB), Mali, West Africa
2) National Institute for Research on Traditional Medicine and Pharmacopoeia (NIRTMP), Mali, West Africa

Pharmacognostic profile of *Zanthoxylum Zanthoxyloides* (Lam.) Zepern. & Timler (Rutaceae), a plant used in the management of sickle cell disease in Mali

Mahamane Haidara, Daouda L Dembele, Sékou Doumbia and Rokia Sanogo

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Abstract
A survey conducted in Bamako, among sickle cell patients revealed that *Zanthoxylum Zanthoxyloides* was the plant most commonly used by these patients to manage their disease. The aim of this study was to determine the botanical characteristics, physicochemical parameters, and chemical constituents of *Zanthoxylum Zanthoxyloides* root harvested in Mali. The botanical characteristics and physicochemical parameters were determined using standard methods described in the African and European Pharmacopoeias. The chemical constituents were characterized by tube reactions. Microscopic examination showed the presence of suber, sclerotic cells, calcium oxalate prisms, fiber groups containing calcium prisms, punctate xylem and starch grains. Water content was less than 10%. Water extractable substances contents and 70% ethanol extractable substances content were greater than 10%. Phytochemical screening revealed the presence of alkaloids, coumarins, saponins, sterols, triterpenes, and tannins. These data can be used for quality control of future samples of *Zanthoxylum Zanthoxyloides* roots, harvested in Mali.

Keywords: *Zanthoxylum Zanthoxyloides*, Pharmacognostic parameters, Mali, sickle cell disease

Introduction
Sickle cell disease, a global genetic disease, is a serious hemoglobin disorder and the most common genetic disorder in the world [1]. Medicinal plants are a therapeutic remedy frequently used in the symptomatic management of sickle cell disease in Mali [2-4]. Results of a survey conducted in Bamako (Mali), among sickle cell patients (SS, SC and Sβ forms), showed that *Zanthoxylum Zanthoxyloides* (Lam.) Zepern. & Timler was the plant most frequently used by his patients for the management of their disease [5]. *Z. zanthoxyloides, Rutaceae family* [6], is a thorny tree or shrub reaching 8 to 12 m in height, distributed in West Africa, from Senegal to Nigeria [7, 8]. *Z. zanthoxyloides* is a well-known medicinal tree, whose roots, root and stem bark, fruits, and leaves are used. Commonly reported indications are pain, rheumatism, dysentery, fever, and sickle cell disease [9]. Research on the roots of *Z. zanthoxyloides* has led to the development of several improved traditional medicines (ITMs) in West Africa. These include FAGARA from the National Institute for Research on Traditional Medicine and Pharmacopoeia (NIRTMP) in Mali [10] and FACA of the Burkina Faso Health Sciences Research Institute [11]. Despite the development of ITMs based on the roots of *Zanthoxylum Zanthoxyloides*, pharmacognostic studies conducted on samples of *Zanthoxylum Zanthoxyloides* roots harvested in Mali are rare. The aim of this study was to determine the pharmacognostic profile of *Zanthoxylum Zanthoxyloides* roots harvested in Mali, by determining botanical characteristics, physicochemical parameters and chemical constituents.

Materials and Methods
Plant material
Roots of *Z. zanthoxyloides* were collected in August 2017 in the NIRTMP botanical garden in Bamako, Mali. An herbarium specimen of the plant is available in the INRMPT herbarium under No. 2957.

The harvested root samples were dried in the shade for 2 weeks in the NIRTMP botanical drying room and then ground into powder using a branded mill (Forplex Grinder).

Determination of botanical characters

Determination of the organoleptic characteristics of the powder

Organoleptic characteristics (color, odor, texture, taste) were determined using the sense organs. Determination of color was carried out by the naked eye; the odor by bringing a sample to the nostrils, the taste by placing a small amount of sample on the tip of the tongue and the texture by touching the dry and wet sample between the fingers [12].

Microscopic examination of the powder

A small amount of the powder was mounted between slide and coverslip either in an aqueous solution of potassium hydroxide (KOH) diluted to 5%, or in a solution of Gazet and Chatelier's reagent. Microscopic elements were observed with the 40 objective of an OPTIKA ITALY microscope and then photographed using an iPad device which was directly connected to the microscope [13, 14].

Physicochemical analyses

Determination of water content

Two grams of powder were introduced into three previously tared crucibles. Samples were dried under study at a temperature of 105 °C for 24 hours. Crucibles were cooled in a desiccator and weighed. Masses obtained were used to calculate the mass loss and to calculate the water content of the powder expressed as a percentage [12, 14].

Determination of total ash

Samples of powders, freed from water and dried during the water content determination, were calcined at 600 °C for 6 hours. After cooling in a desiccator, the ash was weighed. Quantities obtained were used to determine the ash masses and calculate the total ash content, expressed as a percentage [12, 14].

Determination of ash insoluble in hydrochloric acid

Total ash obtained was taken up with 20 mL of 10% hydrochloric acid. Mixture was brought to a boil in a bain-marie for 15 minutes. Solution obtained was filtered through an ashless filter. The residue collected on an ashless filter was placed in a previously tared crucible and calcined at 600 °C for 6 hours. Crucible containing the ash was cooled in a desiccator. Mass of ash insoluble in hydrochloric acid was expressed as a percentage [12, 14, 15].

Determination of water-extractable substances

Place 2 g of powder in an Erlenmeyer flask, then add 40 mL of distilled water and bring to a boil for 15 minutes. Allow to cool and filter. Filtrate obtained was placed in a previously tared glass bottle and evaporated to dryness in an oven. Mass of dry extract obtained was used to calculate the percentage of water-extractable substances [14, 16].

Determination of 70% ethanol-and ether-extractable substances

Place 2 g of powder in an Erlenmeyer flask then add 40 mL of 70% ethanol or ether and to macerate for 24 hours at room temperature in the laboratory then filter. Filtrate obtained was placed in a previously tared glass bottle then evaporated to dryness in an oven. Mass of dry extract obtained was used to calculate the percentage of 70% ethanol-and ether-extractable substances [14, 16].

Phytochemical screening

A preliminary phytochemical screening of the main chemical groups was carried out by tube reactions using conventional reagents. Chemical groups investigated were alkaloids (Dragendorff reagent), anthocyanins (acid and base reaction), anthraquinones (Bornträger reaction), flavonoids (Cyanidine reaction), saponins (foaming test), tannins (trichloride reaction), and sterols and triterpenes (Lieberman reaction) [17]. The results were categorized as follows:

- Strongly positive reaction: +++
- Moderately positive reaction: ++
- Weakly positive reaction: +
- Negative reaction: -

Results and Discussion

Botanical characteristics

Root powder of *Z. zanthoxyloides* had a yellowish color, a pungent taste, an uncharacteristic odor, and a fine texture with a very soft touch and may clump if exposed to moisture. Microscopy of the root powder mounted between a slide and a coverslip in 5% aqueous potassium hydroxide (KOH) solution showed the presence of suber (Figure 1A), sclerotic cells (Figure 1B), calcium oxalate prisms (Figure 1C), and fiber groups containing calcium prisms (Figure 1D). Microscopy of the root powder mounted between a slide and a coverslip in Gazet and Chatelier's reagent showed the presence of starch grains (Figure 1E) and punctate xylem (Figure 1F). These data confirm the information reported in the African Pharmacopoeia published in 2014 [18]. Botanical characteristics (organoleptic characteristics and microscopic elements) will contribute on the one hand to the identification of the powder of the roots of *Zanthoxylum Zanthoxyloides* and on the other hand to the determination of falsifications.

Physicochemical parameters

Physicochemical analysis is an essential element in the quality control of plant material. Results of the physicochemical parameters studied are presented in Table I. The water content was less than 10%. This result is within the limit generally recommended by the Pharmacopoeias [13], which would minimize the risk of proliferation of bacteria and fungi (yeasts and molds) in this powder during its storage [13, 19]. Total ash content was 6.02±0.43%. This value is slightly higher than that reported in the African Pharmacopoeia which recommends a total ash content of less than or equal to 5% for *Z. zanthoxyloides* root powder [18]. Ash insoluble in hydrochloric acid content was less than 1%. This result is within the limit recommended by the African Pharmacopoeia which recommends an ash insoluble in hydrochloric acid content less than or equal to 3.5% [18]. A relatively low ash insoluble in hydrochloric acid content suggests that the powder of the roots of *Z. zanthoxyloides* contains a very small quantity of siliceous elements such as sand and dust [13, 19, 20]. High total ash content combined with low ash insoluble in hydrochloric acid content suggests that *Z. zanthoxyloides* root powder is a good source of minerals compared to cereals, dairy products, some fruits and vegetables [21]. Contents of water- and 70% ethanol-extractable substances were greater than 10% (see Table I). These values are within the limits recommended by the African Pharmacopoeia, which recommends contents of water- and 70% ethanol-extractable substances greater than or equal to 10% [18].

Contents of water- and 70% ethanol-extractable substances were higher than those extracted with ether. These results suggest that the phytochemical constituents of *Z. zanthoxyloides* root powder are more soluble in polar solvents than in apolar solvents.

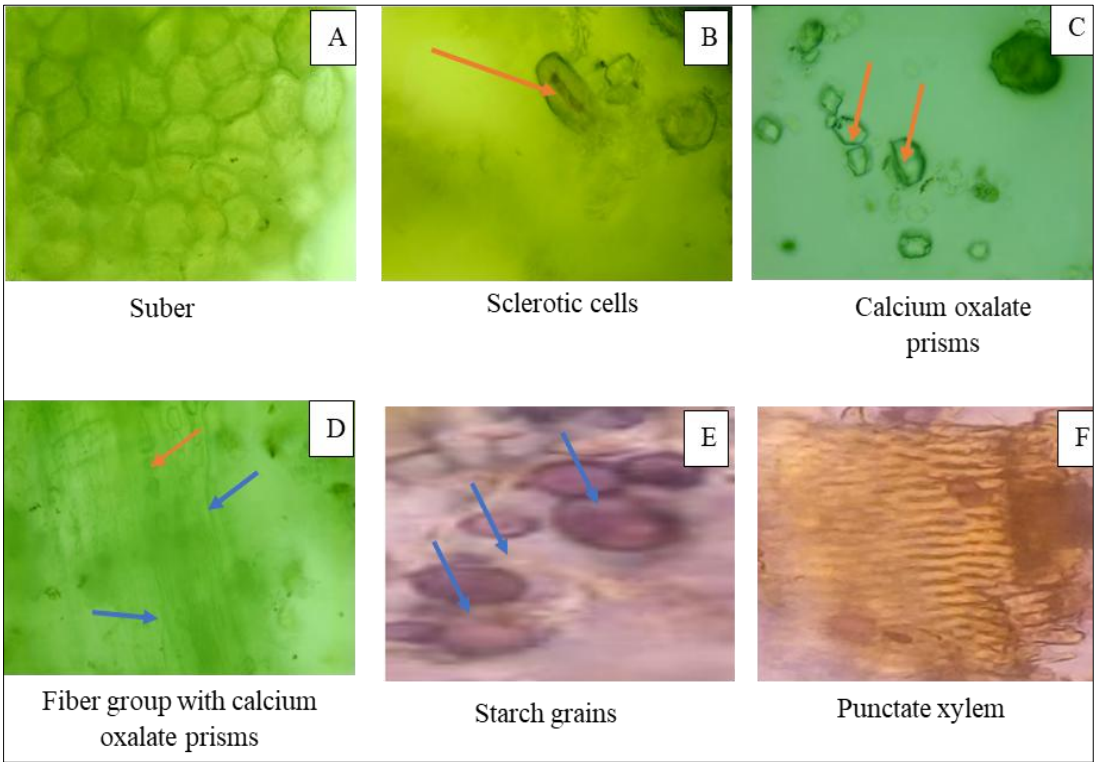

Fig 1: Microscopic elements of root powder (Magnification X 40)

Table 1: Physicochemical parameters of *Z. zanthoxyloides* root

Physicochemical parameters	Value (%)
Water content	8.58±0.14
Total ash	6.02±0.43
Ashes insoluble in hydrochloric acid	0.72±0.03
Water-extractable substances	11.05±0.5
70% ethanole extractable substances	10.9±0.4
Ether-extractable substances	3±0

Characterized chemical groups

Phytochemical screening revealed the presence of alkaloids, carbohydrates, coumarins, saponins, sterols, triterpenes and tannins in the powder of the roots of *Z. zanthoxyloides* (see Table II). These results are slightly different from previous work carried out on samples of *Z. zanthoxyloides* collected in Mali [4, 22]. In these studies, the authors highlighted the presence of flavonoids in the roots of *Z. zanthoxyloides*. In addition, these authors did not find the presence of saponins. These differences could be due to the origin of the *Z. zanthoxyloides* root samples. Indeed, our samples were collected from *Z. zanthoxyloides* cultivated in the NIRTMP botanical garden located in Bamako, while those of Cissé *et al*. [22], ET Togola *et al*. [4] were collected from spontaneous plants of *Z. zanthoxyloides* respectively in Yanfolila and Ouéléssébougou for Cissé *et al*. [22] and Banankoro for Togola *et al*. [4].

Table 2: Chemical groups characterized in the roots of *Z. zanthoxyloides*

Chemical groups	Roots
Alkaloids	++
Anthraquinones	-
Carbohydrates	+++
Coumarins	++
Flavonoids	-
Leucoanthocyanins	-
Saponins	++
Sterols et triterpenes	+++
Tannins	+++

Conclusion

This work has made it possible to determine the botanical, physicochemical and phytochemical parameters of the powder of the roots of *Zanthoxylum Zanthoxyloides*. These data can be used for the quality control of future samples of the roots of *Zanthoxylum Zanthoxyloides*, collected in Mali. However, it would be necessary to repeat this work on samples from several localities in Mali.

Conflict of Interest:
No conflict of interest

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