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Selenium content, antibacterial, antioxidant and anti-sickling activities of *Zanthoxylum gilletii* (De Wild) P.G. Waterman (*Rutaceae*)

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ABSTRACT

Aim: The aim of this study was to identify bioactive compounds, to determine the mineral content and to evaluate the antibacterial, antioxidant and anti-sickling activities of different parts of *Zanthoxylum gilletii*.

Methods: Phytochemical composition was evaluated by general tests as well as chromatographic technics (TLC and HPLC), the mineral micronutrient content was quantified by spectroscopy ICP-OES. The antioxidant activities of the infusions extracts from leaves, stem bark and root bark of *Z. gilletii* were evaluated using ABTS an DPPH assays, the antibacterial activity against four bacteria strains using the micro-dilution method; and the anti-sickling activity was assessed by the Emmel test.

Results: Phytochemical screening revealed the presence of polyphenols such as anthocyanins and flavonoids (stem bark) while stem and root barks contained tannins. Alkaloids were found in the leaves, saponins in leaves, stem and root barks. Leaves and root bark also contained triterpenoids and steroids, while only stem bark contained quinonic derivatives. For phenolic acids and flavonoids, stem and root barks could contain luteolin, chlorogenic acid, caffeic acid and only stem bark could contain rutin. Mineral analysis revealed the presence of macronutrients and micronutrients including calcium, iron, zinc and selenium. All aqueous extracts displayed high ABTS and DPPH radical-scavenging activities at the concentration range of 1–25 ug/mL. The *in vitro* Emmel test showed that the aqueous extracts of the different parts had anti-sickling properties at the concentration of 10.42 µg/mL, 20.83 µg/mL, 83.30 µg/mL for the stem bark, the leaves and the root bark respectively. The stem bark was the most active extract. The results of antibacterial activity test indicated that the all extracts exhibited the highest activity against *Staphyloccocus aureus, Escherichia coli, Enterococcus spp* and *Pseudomonas aeruginosa.* Stem barks showed moderate activity *against P. aeruginosa and* root barks against *S. aureus* and *Enterococcus spp* respectively.

Conclusions: The bioactivities of the different parts could be attributed to alkaloids, phenolic compounds and terpenes. Stem bark showed the best antioxidant, antibacterial and anti sickling activities. *Z. gilletii* contains the phytochemicals that validate its use in Traditional Medicine for the management of sickle cell disease.

Keywords: Micronutrients, Selenium, Sickle cell disease, *Zanthoxylum gilletii*.

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INTRODUCTION

Sickle cell disease (SCD) is a very serious genetic disease linked to the presence of S haemoglobin in the blood and it is a major public health problem in endemic areas such as the Democratic Republic of Congo (DR Congo). To the best of our knowledge, there is no effective medication for this haemoglobinopathy.[1] Traditional Medicine (TM) could occupy a central position in the management of common diseases, including sickle cell disease. Thus, by combining ancestral knowledge from TM and modern scientific techniques derived from biology, chemistry, pharmacology, etc., the African researcher is able to develop new plant-based pharmaceuticals for better management of common neglected diseases such as sickle cell disease.[2] Moreover, it is now recognized that Africa's economic development will depend mainly on science, technology and innovation.

In order to scientifically validate empirical knowledge, to document and preserve it, our research team has been conducting rigorous scientific studies for more than ten years on the plants used in TM

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to treat sickle cell disease, with the aim of improving the country's health coverage.[2] In fact, it has been reported that in the Democratic Republic of Congo, 12% of children hospitalized in pediatric wards are sickle cell disease patients and the annual cost of treatment is estimated to be more than USD 1.000 per patient. This cost is difficult to bear for the majority of the population whose average income is less than USD 2 per day and who for primary health care needs to turn mainly to the use of traditional recipes from medicinal plants.[3] Therefore, the search for active ingredients from natural products is more relevant than ever. The plant kingdom constitutes an invaluable reservoir of biologically active compounds. Among these natural phytochemicals, phenolic and triterpenic acids as well as anthocyanins from Congolese plants have been reported in the literature as having anti-sickle cell properties.[4,5]

Although several studies have demonstrated the anti sickle cell properties of some Congolese botanical species,[4,5] an extensive investigation is needed to make a good use of other plants species such as *Zanthoxyllum gilletii*. *Zanthoxylum gilletii* (De wild) P.G. Waterman (Rutaceae) called *Nkonko, Nkumanga, Nungu nsende* or African satinwood is traditionally used to treat nausea, toothaches, urinogenital complaints including infections.[6,7] *Zanthoxylum gilletii* is a monoecious species distributed between tropical rainforest and wooded savannah. Previous biological and pharmacological studies reported that the *Zanthoxylum* species such as *Zanthoxyllum gilletii* have interesting bioactivities including larvicidal, analgesics, anthelminthic, antiviral, antioxidant, antifungal, antibacterial, anti-inflammatory and analgesic, antiplasmodial and cytotoxicity activities.[7,8]

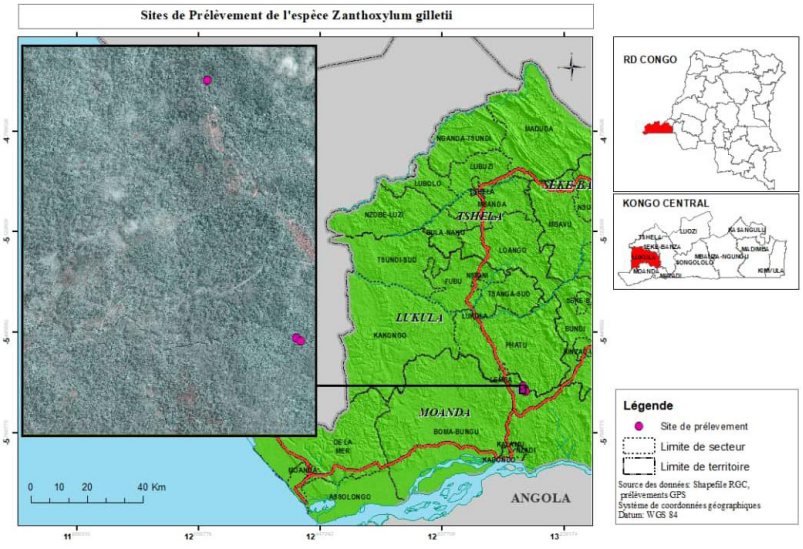

Figure 1 Harvested site of *Zanthoxylum gilletii* (De wild) P.G. Waterman (*Rutaceae*)

In order to valorize the use of Congolese *Zanthoxylum gilletii* in the management of sickle cell disease, this study was conducted to evaluate the qualitative phytochemical composition of this plant by general reactions, and chromatographic technics, the mineral composition by ICP-OES and the antioxidant activities using ABTS an DPPH assays, the antibacterial activity against four bacteria strains using the micro-dilution method; and the *in vitro* anti-sickling activity by the Emmel test.

MATERIAL AND METHODS

Plant Material

The plant material consisted of leaves, stem bark and root bark of *Zanthoxylum gilletii* (De wild) P.G. Waterman (*Rutaceae*) harvested in November 2018, in the Luki Biosphere Reserve in Bas-fleuve territory, Kongo Central Province, DRC (Figure 1).

The plant was identified at the Herbarium of the "Institut National d'Etudes et de Recherches Agronomiques" (Voucher n°: INERA/LUKI 1121) and confirmed by the" Laboratoire de Botanique systématique et Ecologie des plantes", Department of Biology, Faculty of Sciences, University of Kinshasa. The samples were dried at room temperature (± 27 °C) in the Molecular Bio-Prospecting Laboratory (Department of Biology) for two weeks and ground to a fine powder.

Chemical analysis

Phytochemical Screening

Phytochemical screening of *Z. gilletii* was performed according to Bruneton.[9] Analytical TLC of 10 µL of solution for 10 mg/mL of methanolic and dichloromethane extracts solutions was carried out on normal phase Silica gel 60 F_{254} plates (Merck), using different eluents for the identification of secondary metabolites.[10] Analytical separation on HPLC-DAD was carried out on a Hypersil ODS® RP18 column as described by Kapepula et al. (2017).[11]

Mineral analysis by ICP-OES

The determination of minerals content was carried out by the method of water and nitric acid and analysis by Inductively Coupled Argon Plasma Optical Emission Spectrometry (ICP-OES).[12] 0,3 g of plant powder diluted in 5mL of distilled water was placed in PM60 Teflon bombs (Analytikjena 40Bar) and heated at 60°C, then 10 mL of nitric acid (65% HNO_3) (Merck) were added. The mixture was allowed to react for 30 minutes at room temperature in the bombs were covered with caps and then stripped with HNO_3 / H_2O (v / v, 1: 1). The bombs are then placed in the high frequency microwave mineralizer (Analytikjena AG TOPwave: 2.5 Ghz, Germany) controlled by microcomputer by choosing the Vegetable Leave

mode as a digestion mode at 180°C, 50bar for 1 hour. At the end of mixing, the digester is stopped by letting the bombs rest for 3 hours until completely cooled. The cooled analyte is thus carefully transferred from bombs by filtration on Whatman filter paper, to 50 mL volumetric flasks previously stripped. The initial volume was diluted to 50 mL with distilled water and 13 mL were placed in 15 mL conical flask previously stripped for reading by Inductively Coupled Argon Plasma Optical Emission Spectrometry (ICP-OES) (Optima 8300 Perkin Elmer, USA). The analysis was performed in triplicate. The calibration of the ICP-OES is performed using the working standard prepared from the commercially available standard multielement solution 3 at two points (1mg / L and 2.5mg / L, Perkin Elmer, USA). The most appropriate wavelength, gaseous argon flow, plasma stabilization and other ICP-OES instrument parameters for minerals are selected and measurements are made in the linear range of the working standards used for calibration.

Working conditions were: Instrument: ICP-OES (Optima 8300 Perkin Elmer, USA); Power of Rf: 1500Watt; Plasma gas flow (Ar): 8L /min; Nebulizer: 0.70L / min; Auxiliary gas flow (Ar): 0.2L / min; Viewing size: 5-22 mm; Copy and playback time: 1-5s (maximum 45s); Flow time: 1s (maximum 10s); View: Radial.

Antioxidant activity

The extracts were solubilized in the mixture DMSO-Water (1:1), thus their effect was compared to a control test performed with the mixture alone. Antioxidant activity was assayed through spectrophotometric ABTS and DPPH that were performed according to the method described by Kapepula et al. (2017).[11]

Antibacterial Activity

The antibacterial activity of *Zanthoxylum gilletii* (De Wild) P.G was assessed against *Staphylococcus aureus* (*S. aureus* ATCC 25923), *Escherichia coli* (*E. coli* ATCC 29922), *Pseudomonas aeruginosa* (*P. aeruginosa* ATCC 9027) strains and *Enterococcus* spp, by the micro-well dilution method and the minimum inhibitory concentration (MIC) values, which represent the lowest sample concentrations that completely inhibit the growth of microorganisms were obtained using this method.[13]

The 20 mg of extracts were each dissolved in DMSO (250 µL) and diluted with Mueller–Hinton Broth (MHB) in order to reach concentrations of 2000 µg/mL and a 5 ml solution (final volume, and 5% DMSO final concentration). These solutions were used as stock solutions. The inocula of microorganisms were prepared from 24 h old MHB cultures. The microbial suspensions were prepared by adding five colonies of each of the test bacteria to 2 mL of with sterile physiological solution (0.9% NaCl) and adjusted with this sterile physiological solution to match that of a 0.5 McFarland standard solution (108 cells/ml). They were then diluted (1/100) to achieve 10^6 CFU/mL. The assay was carried out using sterile clear polystyrene 96 well microtiter plates (round bottom). The wells in the columns 2 to 8 and those in columns 11 and 12 were filled with 100 µL MHB (Mueller Hinton Broth). Briefly 200 µL of stock solution of each *Zanthoxylum* extracts were added to the wells in column 1 (A1 to H1), and two-fold serial dilutions were made from column 1 to column 8. Then 5 µL of the inoculum were dispensed to all the wells except those in column 12. The wells in columns 11 and 12 we used as positive and negative controls. The negative control wells (growth control) contained MHB and bacteria suspension without test sample (column 11) and the positive control wells contained only MHB (control of MHB sterility: column 12). The microplates (96 wells) were incubated at 37°C for 24 hours. After the incubation, 5µL de colorant resazurin 1% (7-Hydroxy-3H-phenoxazin-3-one 10-oxide) were added to each well and the microplates were then incubated for 5 hours. The minimum inhibitory concentration (MIC) was determined as the lowest aqueous extract concentration at which no growth was observed after 24 and 48 hours. All experiments were performed in triplicate.

Anti-sickling cell activity

The blood samples used to evaluate the antisickiling activity of the plant extracts were provided by the "Centre de Médecine Mixte et d'Anémie SS de Kinshasa" in the DRC. None of the patients had been recently transfused with Hb A blood and all experiments were performed with freshly drawn blood. In order to confirm their SS status, the above blood samples were first characterized by hemoglobin electrophoresis on cellulose acetate gel (at alkaline pH) and then stored at ± 4°C in the refrigerator. Consent forms were obtained from all patients participating in the study and research procedures were approved by the Ethics Committee of the Department of Biology of Faculty of Sciences (University of Kinshasa). The Emmel test was performed to evaluate the capacity of positive control and extracts to correct the sickling of red blood cells as followed: An aliquot of SS blood was diluted with 150 mM physiological phosphate buffered solution and mixed with an equivalent volume of 2% sodium metabisulfite. One drop of the mixture was placed on a microscope slide with or without plant extracts and covered with a slide. Paraffin was applied to

completely seal the edges of the microscopic preparation to exclude air (hypoxia). Triplicate analyses were performed for each extract. Red blood cells (RBCs) were analyzed using computer-assisted image analysis software (Motic Images 2000, version 1.3; Motic China Group Co LTD). Betulinic acid was used as positive control.[14]

Statistical Analysis

Each concentration was tested in triplicate in each assay, and at least 3 different assays were performed. All results were expressed as mean values ± standard deviation (SD). Statistical data analysis was processed using Microcal Origin 8.5 Pro software.

RESULTS

Phytochemicals

Phytochemical screening of *Z. gilletii* revealed the presence of polyphenols such as anthocyanins and flavonoids in all parts while stem and root bark contained tannins. Alkaloids were found only in the leaves and saponins in the leaves, the stem and the root barks. Leaves and root bark also contained triterpenoids and steroids, while only stem bark contained quinonic derivatives. TLC analysis revealed the presence of terpenes, polyphenols such as phenolic acids and flavonoids (Figure 2 and Figure 3).

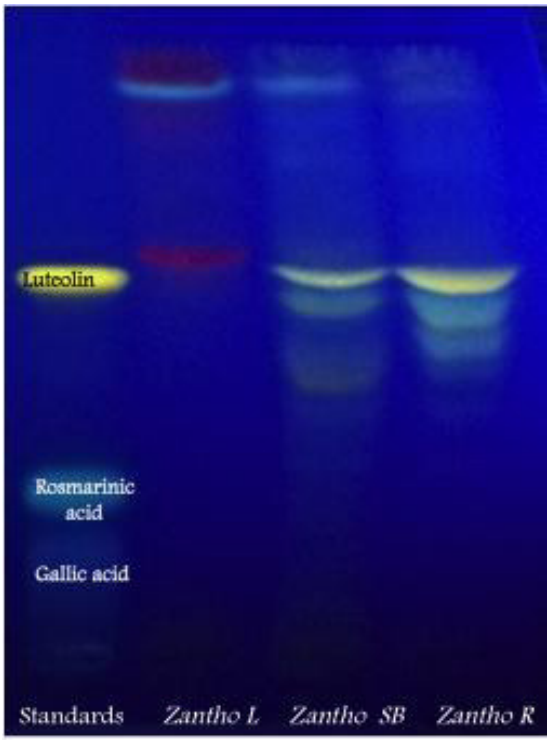

Figure 2 TLC chromatogram of methanolic extracts from parts of *Z. gilletii* (Leaves: Zantho L, Stem bark: Zantho SB and Root bark: Zantho R) with gallic acid, rosmarinic acid and luteolin as standards; developed with dichloromethane/acetone/formic acid (85:20:10; v/v/v) and visualized at 365 nm with Natural Products-PEG reagent. Flavonoids are detected as yellow fluorescent spots and phenolic acids as blue fluorescent spots

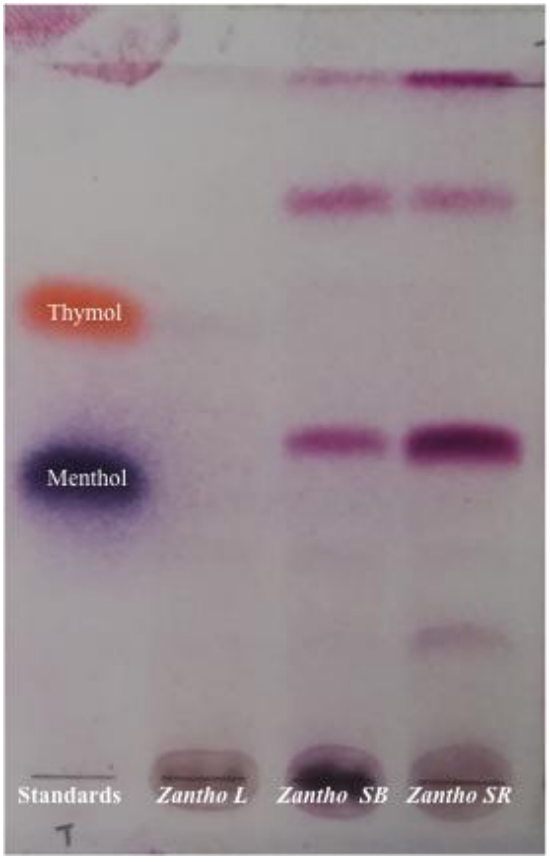

Figure 3 TLC chromatogram of dichloromethane extracts from parts of *Z. gilletii* (Leaves: Zantho L, Stem bark: Zantho SB and Root bark: Zantho SR) with oleanolic acid, menthol and thymol as standards; developed with toluene/ethyl acetate (93:7; v/v) and visualized at visible with sulfuric anisaldehyde reagent. Terpenes are detected as violet spots

By comparison with used standards, the chromatographic fingerprints show the presence of glycosylated flavonoids and phenolic acids (caffeic acid, chlorogenic acid, luteolin and rutin) as main compounds for stem and root barks (Figure 4).

Fingerprints of leaves are completely different from those of stem and root barks. HPLC fingerprints indicated the presence in the leaves of four major compounds and one minority compound having a retention times corresponding to rutin (23.722 min) and caffeic acid (7.617 min) and three

Table 1 **Concentration of macronutrients expressed in mg/kg (ppm) of dry matter (Mean ± SD, n=6)**

	Concentration (mg/kg)		
Mineral Nutrients	***Leaves***	***Stem bark***	***Root bark***
Potassium	5596 ± 58	773 ± 7	1044 ± 32
Sodium	971 ± 40	861 ± 18	932 ± 60
Phosphore	1910 ± 70	896 ± 66	925 ± 79
Calcium	8584 ± 78	11743± 68	9696 ± 65
Magnesium	3997± 51	658 ± 7	1160 ± 43

Table 2 **Concentration of micronutrients expressed in mg/kg (ppm) of dry matter (Mean ± SD, n=6)**

	Concentration (mg/kg)		
Mineral Nutrients	***Leaves***	***Stem bark***	***Root bark***
Iron	190.2 ± 8.2	362.6 ± 12.6	1982.2 ± 122.8
Manganese	244.6 ± 7.7	146.1 ± 4.2	104.3 ± 44
Zinc	23.3 ± 12.2	13.8 ± 2.5	30.8 ± 8.7
Cupper	24.2 ± 8.7	19.8 ± 0.6	21.5 ± 0.9
Chrome	28.5 ± 6.6	6.9 ± 0.8	6.4 ± 1.26
Selenium	492.8 ± 48.3	784.5 ± 60.6	997.8 ± 90.4
Cobalt	31.2 ± 6.1	19.1 ± 5.9	10.7 ± 2.2

Table 3 **IC_{50} values (µg/mL) of infusion extracts of parts of *Z. giletti* on ABTS and DPPH assays (Mean ± SD, n = 6)**

	CI_{50}	
Samples	**ABTS (µg/mL)**	**DPPH (µg/mL)**
Zanthoxylum Leaves	16,71 ± 0,99	22,38 ± 1,28
Zanthoxylum Stem Bark	9,14 ± 1,5	16,4 ± 2,28
Zanthoxylum Root Bark	8,22 ± 1,1	20,6 ± 2,2
Quercetin	1.42 ± 0.04	3.21 ± 0.99

Table 4 **Minimum inhibitory concentrations MICs (µg/mL) of infusions extracts from diffrent part of *Zanthoxyllum gilletii* (data from three experiments in triplicate, MIC defined as the lowest concentration for which no growth was observed in every tested well)**

	IMC* values (µg/mL) of microorganism			
Samples	***Escherichia coli***	***Staphyloccus aureus***	***Pseudomonas aeruginosa***	***Enterococcus spp***
Leaves	250	125	125	125
Stem bark	62.5	62.5	500	125
Root bark	250	500	250	500

(*) : MIC values below 500 µg/mL : high antibacterial activity ; 500 to 1000 µg/mL weak activity and over 1000 µg/mL inactive[13]

unknown majority compounds. On the other hand, stem bark contains seven major compounds having retention times corresponding to the standard phenolic compounds used: rutin (23.722 min); caffeic acid (7.617 min), quercetin (25.337 min) and chlorogenic acid (18.867 min) and three other unknown compounds. In the end, the root bark presented seven majority compounds with retention times that do not correspond to any standard, while three minority compounds had retention times close to standards: caffeic acid (7.617 min); quercetin (25.337 min) and chlorogenic acid (18.867 min). Stem and root barks could contain luteolin, chlorogenic acid, caffeic acid and only stem bark could contain rutin.

Mineral nutrients

ICP-OES analysis showed the presence in varying proportions of mineral nutrients among which macronutrients and micronutrients, in different parts of the plant (Tables 1 and 2).

The potassium content was very higher in the leaves than in all other parts, but all parts contained less sodium except for the stem bark, where the sodium content is higher than the potassium content. Phosphorus was in proportionally in equal amounts in stem and root barks and was almost the double in the leaves. The calcium content was high in the stem bark, followed by the root bark and the leaves. Magnesium content was low in stem bark, very high in the leaves compared to the root bark due to the presence of leaf chlorophyll. Iron was comparatively very high in root bark but low in stem bark and leaves.

As a micronutrient, manganese was comparatively higher in the leaves than in other parts of the plant. Copper and zinc were in moderate amounts in the different parts of the plant, but low in the stem bark. Although the concentration of chromium is quite low in the stem and root bark of the plant material studied, it is comparatively higher in the leaves. Selenium content is higher in root bark than in stem bark and leaves. Cobalt is found in low quantities in the stem and root barks and high in the leaves.

Antioxidant activity

ABTS and DPPH assays showed that infusion extracts from *Zanthoxylum* parts have the ability to scavenge free radicals connected with their IC_{50} values (Table 3).

The stem and root barks were more active than the leaves and the differences were statistically significative ($p < 0,05$). The IC_{50} values of ABTS assay were weak than those of DPPH assay.

Antibacterial Activity

The results of the evaluation of the antibacterial activity of *Zanthoxylum* infusions are shown in Table 4.

Table 5 **Values of the radius, surface area and perimeter of SS erythrocytes before and after treatment with 10.42 µg/mL of *Z. gilletii* stem bark extract (Mean ± SD, n=6)**

Mesuread parameters	None treated SS Erytrocytes	Treated SS Erytrocytes	Observations
Radius (µm)	-	10.0 ± 0.6	Reappearance
Surface (µm²)	297.1 ± 0.8	328.6 ± 0.13	Increase
Perimeter (µm)	115.7 ± 0.5	60.4 ± 0.11	Decrease

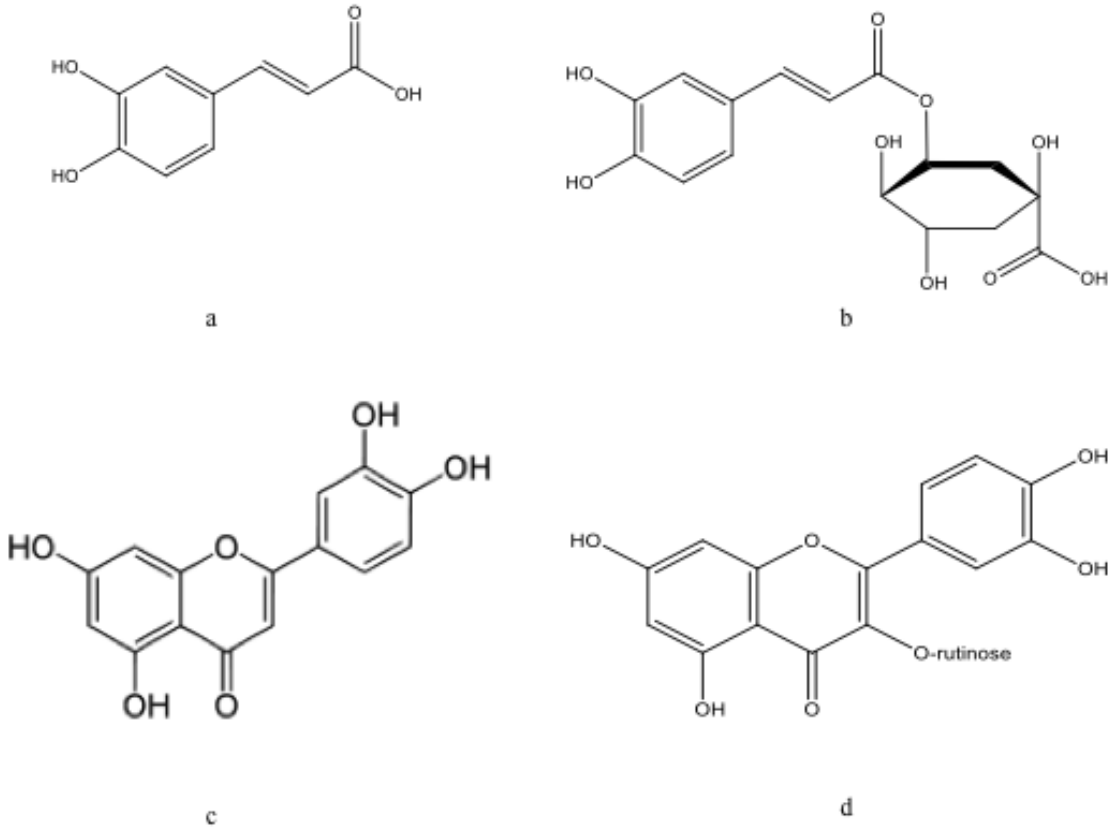

Figure 4 Chemical structures of caffeic acid (a), chlorogenic acid (b), luteolin (c) and rutin (d)

All extracts showed effective antibacterial activities towards the tested strains. Infusions extracts from the leaves showed high antibacterial activities against all bacteria strains tested while the stem bark showed high antibacterial activity against *Staphylococcus aureus*, *Enterococcus spp and Escherichia coli* and moderate activity against *Pseudomonas aeruginosa*. Infusions extracts from root bark were found to have moderate activity against *Staphylococcus aureus* and *Enterococcus spp*.

Anti-sickling activity

The figure 5 shows that in the absence of plant extracts, the erythrocytes are sickle-shaped, confirming the sickle-cell nature of the blood sampled. However, in the presence of the infusion extracts, the sickle cell erythrocytes revert to the normal biconcave form, showing the effects of extracts from different parts of *Z. gilletii* on the morphology of erythrocytes at the concentration of 10.42 µg/mL, 20.83 µg/mL, 83.30 µg/mL for the stem bark, the leaves and the root bark respectively.

Table 5 gives the calculated mean values of the radius, surface area and perimeter of SS erythrocytes before and after treatment with 10.42 µg/mL of *Z. gilletii* stem bark extract. At this concentration extract from stem bark showed a high anti-sickle cell activity.

DICUSSION

Phytochemical screening of *Z. gilletii* revealed that the chromatographic fingerprints of different parts were characteristic. Fingerprints of leaves are completely different from those of stem and root barks. Previous phytochemical studies conducted on some species of the genus *Zanthoxylum* have also revealed the presence of alkaloids, saponins, tannins, reducing sugar, phenols, anthraquinones, cardiotonic glycosides, resins as common

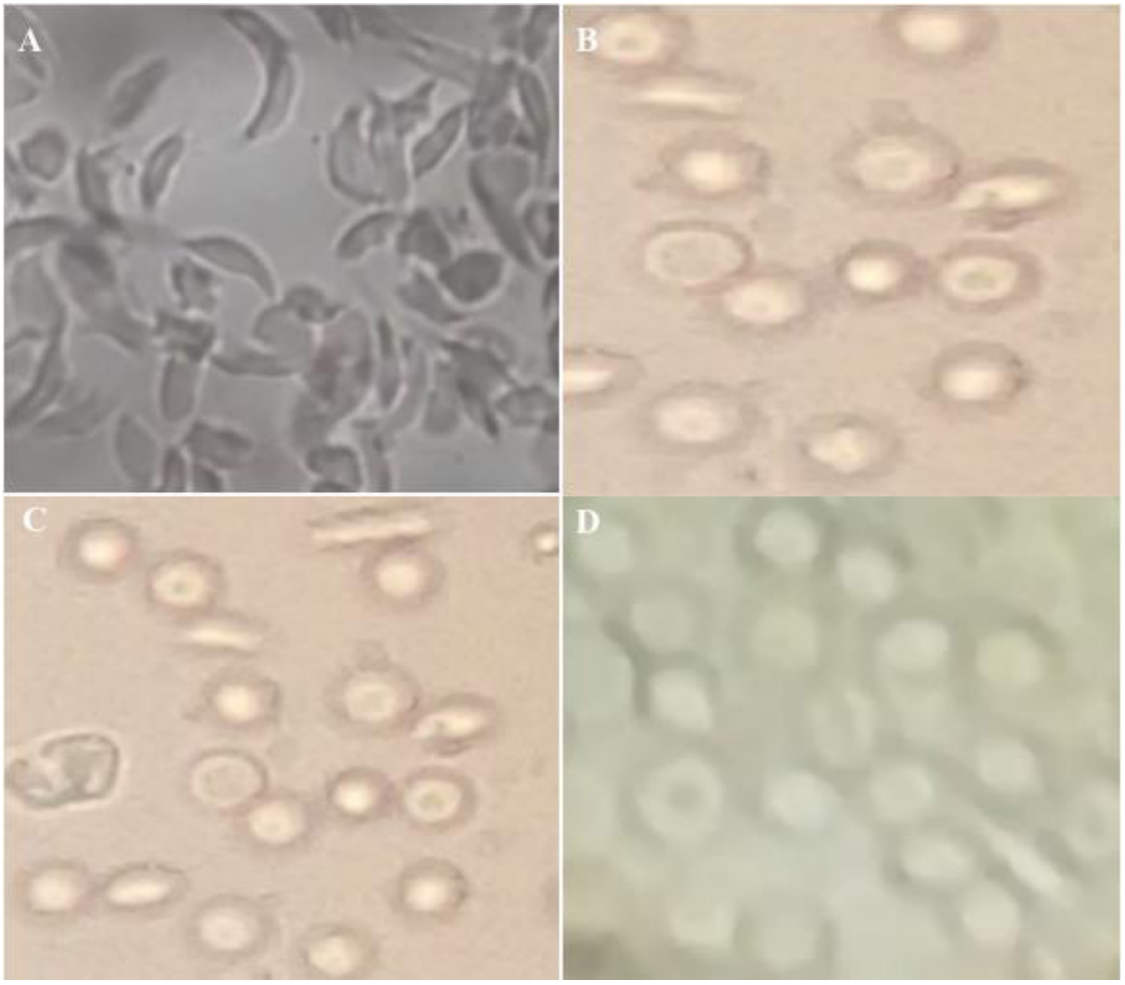

Figure 5 Morphology of non treated erytrocytes (A); treated erytrocytes with 20.83 µg/mL of leaves extract (B); treated erythrocytes with 10.42 µg/mL of stem bark extract (C); treated erytrocytes with 83.3 µg/mL of root bark extract (D) from *Z. gilletii* [X 500; NaCl 0.9%; $Na_2S_2O_5$ 2%]

secondary metabolites. *Zanthoxylum*' alkaloids are of various skeletal types including: benzophenanthridine, protoberberine, bishordeninyl, aporphine, amides, coumarins, lignans and are considered to have a chemotaxonomic importance to the genus. Other metabolites such as flavonoids, sterols and terpenes have also been isolated from plants of this genus.[8,9,10,15] Sadeer et al. (2019) showed that *Z. gilletii* contains quercetin, luteolin, hesperidin, diosmin and some caffeoylquinic acids among which caffeic acid, chlorogenic acid an rutin would be present in our samples. Further studies are needed to confirm the identity of these compounds knowing that chemical profile is generally influenced by the harvesting season, the pH of soils, the choice and stage of drying conditions, the geographic location, chemotype or subspecies, and choice of part plant or genotype or extraction method.[7] Plants require macro- and micronutrients, each of which is essential for a plant to complete its life cycle and their availability can fluctuate greatly in both space and time due to environmental factors such as weather, climate and physicochemical properties.[16] Mineral nutrients are required to meet a wide variety of essential metabolic and structural functions in the human body and their requirements and metabolism can be altered by chronic diseases.[17,18] Potassium is an electrolyte, which, along with other substances, regulates the hydroelectrolytic balance of the body, participates in maintaining tissue excitability and in the transmission of nerve impulses. Due to the solubility of its salts, sodium plays an important role in the transport of metabolites. Phosphorus plays an important role in the human body almost all the phosphorus is combined with oxygen to form phosphate (which is a major component of nucleic acids, for instance). Calcium makes up a large proportion of bone, human blood and extracellular fluid; it is necessary for the normal functioning of the heart muscles, for blood and milk coagulation, and for the regulation of cell permeability. In humans, Magnesium is required in plasma and extracellular fluid, where it helps

to maintain osmotic balance and participates in many reactions as a cofactor of enzymes. Iron is a very important element since it is essential for the formation of hemoglobin. Copper is also a component of many enzymatic systems such as cytochrome oxidase and ceruloplasmin.[17,18] Zinc is a component of many metalloenzymes, including some enzymes that play a central role in nucleic acid metabolism.[18] In addition, Zinc is a membrane stabilizer and stimulator of the immune response. Cobalt enters in the structure of vitamin B12 or cobalamin. The activities of cobalt are those of vitamin B12, in the production of red blood cells and regulation of the functioning of various enzymes. Chromium plays a vital role in carbohydrate metabolism and its deficiency leads to diabetes. Chromium deficiency leads to hyperglycaemia, growth retardation, neuropathy, cataract and atherosclerosis. Selenium is an essential micronutrient in all-human tissues. It works together with vitamin E as an antioxidant. It protects cells from damage caused by radical species. Selenium may help protect against diseases associated with oxidative damage, including cancers and sickle cell anemia.[17,18] Mineral composition of *Z. gilletii* especially in antioxidant micronutrients advocates to this plant to offer more advantage in the management of pathologies characterized by perturbation in mineral metabolism. Delesderrier et al. (2019) suggested that the nutritional care protocols for patients with SCD should include dietary sources of selenium in order to reduce the risk of hemolysis. Sickle cell disease (SCD) is a genetic hemoglobinopathy characterized by chronic hemolysis that is promoted by increased oxidative stress.[19] All infusion extracts from different parts of *Z. gilletii* exhibited higher scavenging activities but lower than quercetin, used as positive control. *Z. gilletii* displayed good antioxidant activities with IC_{50} values inferior to 25 µg/mL. The IC_{50} values obtained in ABTS assay were weak than those in DPPH assay. DPPH assay detected only hydrophilic antioxidants in the contrary of ABTS assay, which detected hydrophilic and lipophilic antioxidants in the analysed matrice.[11] To the best of knowledge, few studies reported the antioxidant activities of *Z. gilletii*. However Sadeer et al. 2019, reported that the extract from stem bark of *Zanthoxyllum gilletii* was better scavenger than leaf extract.[7] Phenolic compounds could be major contributors to antioxydant activity of infusions of *Zanthoxyllum* parts. So, it is possible also that antioxidant micronutrients such as zinc, selenium could contribute to their activity. Sickle cell disease (SCD) is associated with oxidative damage related to deficiency of plasma antioxidants. Chronic oxidative stress play a significant role in the development of microvascular dysfunction, damage to cellular macromolecules and multiple organ dysfunction.[20] *Zanthoxyllum gilletii* could contribute to reduce the effects of oxidative damage by their antioxidant potential. SCD is typified by painful vaso-occlusive episodes, haemolytic anaemia and organ damage. A secondary complication is infection, which can be bacterial, fungal or viral.[21] Infection remains a main cause of overall mortality in patients with SCD in low- and middle-income countries due to increased exposure to pathogens, increased co-morbidities such as malnutrition, lower vaccination rates, and diminished access to definitive care, including antibiotics and blood.[21] *Streptococcus pneumoniae* have been identified as important cause of the majority of cases of severe sepsis, followed by *Neisseria meningitidis*, *Haemophilus influenzae*, and *Escherichia coli*. Chronic leg ulcers are a frequent and debilitating complication of SCD. Infection is a complication, rather than a cause, of leg ulcers. Secondary infection of ulcers may occur spontaneously or be a result of trauma. The most commonly isolated bacteria are *Staphylococcus aureus, Pseudomonas, Streptococci, or Bacteroides*.[22] The antimicrobial activity of infusion extracts against all the strains tested could be explained by the presence of phytochemicals such as phenolic compounds and/or alkaloids.[13] Leaves and stem bark showed the higtest antibacterial activities. Extracts from different parts of *Z. gilletii* which proved by their antibacterial activities to be potentially effective can be used as natural alternative to control infections in SDC.

Sickle hemoglobin polymerizes in the deoxygenated state, resulting in erythrocyte membrane deformation.[22] Extracts from *Zanthoxylum gilletii* have shown an antifalcemic effect (anti-sickling activity) on the erythrocytes with the stem bark the most active extracts and this activity could be due to the phenolic and terpenic compounds identified in the plant. Anti-sickling activity of phenolic compounds and triterpenic acids from Congolese plants were largely reported previously.[4,5,12,23-25] Tshilanda et al. (2015) revealed that ursolic acid was the major biologically active compound responsible of antisickling activity of *Ocimum gratissimum*.[24] Phenolic acids such as lunularic acid and rosmarinic acid, anthocyanins showed interesting antisickling activities at low concentrations and could be responsible for this activity in these natural product extracts.[25] The anti-sickle cell activity is reflected at the cellular level by the reappearance of the radius value, the increase in cell surface area and the decrease in its perimeter. Indeed, the

non-treated erythrocytes do not have circular form so the soft could not give the mean radius of red blood cells. In presence of extract, erythrocytes recovered their circular (biconcave) form that conducts to the reappearance of the radius value. Bioactivities of extracts from *Z. gilletii* demonstrate the need for the validation of traditional recipes by scientific evidence.

CONCLUSIONS

The aim of this study was to evaluate the chemical composition, the antibacterial, antioxidant and anti-sickling activities of *Zanthoxylum gilletii*. The results revealed the presence of saponins anthocyanins, flavonoids and tannins in different parts of this plant. Only leaves and root bark contained triterpenoids and steroids while only stem bark contained quinonic derivatives. Phytochemical profiling by TLC and HPLC indicated the likely presence of caffeic acid, rutin, quercetin and chlorogenic acid in different parts of the plant. Mineral analysis revealed high levels of calcium in the stem bark and iron, zinc and selenium in the root bark. Stem bark showed the best antioxidant, antibacterial activity towards *Staphylococcus aureus*, *Enterococcus spp and Escherichia coli* and anti sickling activity. However, further studies should be carried out to characterize the unidentified bioactive compounds, to evaluate their *in vitro* bioactivities as well as to determine in the future especially by *in vivo* studies, to demonstrate the benefit of *Zanthoxylum* extracts on health.

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Availability of data and materials

All data and materials are presented in the manuscript.

Authors' contributions

Authors Florent B. Mukeba, Johnny Mukoko Bopopi and Koto-te-Nyiwa JP. Ngbolua collected, dried, reduced in powder and perfomed the extractions. Florent B. Mukeba, Jérome V. Bamba, Fanfan Kitwa, Manix M. Mayangi, Christian Kikweta and Paulin K. Mutwale did the literature study, participated in experimental works. Johnny Mukoko Bopopi, Nadège K. Ngombe, Pius T. Mpiana interpreted the data, performed statistical analysis and prepared the manuscript. Koto-te-Nyiwa JP. Ngbolua and Théophile F. Mbemba designed the study. Nadège Ngombe K. and Théophile F. Mbemba approved the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

REFERENCES

1. Bongo GN, Inkoto C, Masengo C, Tshiama C, Lengbiye E, Djolu R. and Kapepula M. *et al*. Antisickling, Antioxidant and Antibacterial Activities of *Afromomum alboviolaceum* (Ridley) K. Schum, *Annona senegalensis* Pers. and *Mondia whitei* (Hook. f.) Skeels. Am J. Lab. Med. 2017; 2: 52-59.
2. Muthu C, Ayyanar M, Raja N, and Ignacimuthu S. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J. Ethnobio. Ethnomed. 2006; 2: 43. doi: 10.1186/1746-4269-2-43.
3. Iserin P. Encyclopédie des plantes médicinales. Identification-Préparation-pharmacologie. Etudiants et professionnels paramédicaux. 2001 ; 4eme Edition, pp. 426.
4. Mpiana PT, Kimbadi BL, Ombeni AM, Ngbolua KN, Tshibangu DST, Tshilanda DD *et al*. *In vitro* inhibitory effects and anti-sickle erythrocytes haemolysis of *Dicliptera colorata* C.B. Clarke, *Euphorbia hirta* L. and *Sorghum bicolor* (L.) Moench. Open Journal of Blood Diseases. 2013; 3:43-48.
5. Ngbolua KN, Mpiana PT, Mwanza BF, Tshibangu DST, Tshilanda DD, Masengo AC *et al*. Antisickling and antibacterial activities of *Garcinia punctata* Oliv. (Clusiaceae) and *Tetradenia riparia* (Hochst.) Codd (Lamiaceae) from Democratic Republic of the Congo. J of Advancement in Medical and Life Sciences. 2016, V4I1. DOI: 10.15297/JALS.V4I1.01.
6. Latham P, ku Mbuta AK. Useful plants of Bas-Congo Province, Democratic Republic of Congo. 2nd ed. Canterbury: Mystole Publications. 2006. 1- 276 p.
7. Bibi Sadeer N, Llorent-Martínez EJ, Bene K, Fawzi Mahomoodally M, Mollica A, Ibrahime Sinan K, et al. Chemical profiling, antioxidant, enzyme inhibitory and molecular modelling studies on the leaves and stem bark extracts of three African medicinal plants. J Pharm Biomed Anal. 2019; 174: 19–33.
8. Sinan KI, Zengin G, Bene K, Mahomoodally MF. Chemistry and pharmacology of three antiplasmodial traditional medicinal plants from tropical Africa – A review. South African J Bot. 2019; 126: 265–76. Available from: https://doi.org/10.1016/j.sajb.2019.04.024
9. Bruneton J. Pharmacognosie, Phytochimie et Plantes médicinales. Edition Technique et Documentation-Lavoisier. Paris. 1999; 3e édition, 421- 499.
10. Wagner H, Bauer R, Melchart D, Xioa PG. and Staudinger A. Chromatographic Fingerprint Analysis of Herbal Medicinal: Thin-Layer High Performance Liquid Chromatography of Chinese Drugs. Vol. 3, Springer International Publishing, Berlin. 2013.
11. Kapepula PM, Ngombe KN, Tshisekedi TP., Tsumbu C, Franck T, Mouithys-Mickalad A, Frédérich M, *et al*. Comparison of Metabolic Profiles and Bioactivities of the Leaves of Three Edible Congolese Hibiscus Species. Natural Product Research. 2017; 31, 2885-2892.
12. AOAC (Association of Official Analytical Chemists). *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, DC, USA. 2019; 18th edition.
13. Okusa PN, Penge O, Devleeschouwer M, Duez P. Direct and indirect antimicrobial effects and antioxidant activity of Cordia gilletii De Wild (Boraginaceae). J Ethnopharmacol. 2007; 112(3): 476–481.

14. Mpiana PT, Mudogo V, Nyamangombe L, Kakule MK, Ngbolua KN, Atibu EK, *et al*. Antisickling Activity and Photodegradation Effect of Anthocyanins Extracts from *Alchornea cordifolia* (SCHUMACH & Thonn.) and *Crotalaria retusa* L. Ann. Afr. Med. 2009; 2(4): 240-245.
15. Adesina SK. The Nigerian *Zanthoxylum*: chemical and biological values. African Journal of Traditional, Complementary and Alternative Medicines. 2005; 2(3).
16. Alexou M, Peuke AD. Roles and Functions of Plants Mineral Nutrients. In: Plant Mineral Nutrients. 2013. p. 195–207.
17. Miguel de la Guardia M, Garrigues S. Handbook of mineral elements in food. Handbook of Mineral Elements in Food. 2015. 1–766 p.
18. Cockell KA. Mineral Nutrients. In: Schwab M. (eds) Encyclopedia of Cancer. Springer, Berlin, Heidelberg. 2011. p. 2318-2322.
19. Delesderrier E, Cople-Rodrigues CS, Omena J, Fleury MK, Brito FB, Bacelo AC, et al. Selenium status and hemolysis in sickle cell disease patients. Nutrients. 2019;11(9):1–11.
20. Smith OS, Ajose OA, Adegoke SA, Adegoke OA, Adedeji TA, Oderinu KA. Plasma level of antioxidants is related to frequency of vaso-occlusive crises in children with sickle cell anaemia in steady state in Nigeria. Pediatr Hematol Oncol J [Internet]. 2019;4(1):17–22.
21. Sobota A, Sabharwal V, Fonebi G, Steinberg M. How we prevent and manage infection in sickle cell disease. Br J Haematol. 2015; 170 (6):757–767.
22. Ochocinski D, Dalal M, Black LV, Carr S, Lew J, Sullivan K, et al. Life-Threatening Infectious Complications in Sickle Cell Disease: A Concise Narrative Review. Front Pediatr. 2020; 8(February).
23. Mpiana PT, Ngbolua KN, Mudogo V, Tshibangu DST, Atibu EK, Tshilanda DD *et al*. Antisickle erythrocytes haemolysis properties and inhibitory effect of anthocyanins extracts of *Trema orientalis* (Ulmaceae) on the aggregation of human deoxyhemoglobin S *in vitro*. Journal of Med Sciences. 2011; 11(3): 129-137.
24. Tshilanda DD, Onyamboko DNV, Babady PB, Ngbolua KN, Tshibangu DST, Dibwe EF *et al*. Anti-sickling Activity of Ursolic Acid Isolated from the Leaves of *Ocimum gratissimum* L. (*Lamiaceae*). Nat. Prod. Bioprospect. 2015; 5: 215-221.
25. Tshilanda DD, Onyamboko DVN, Babady PB, Mutwale PK, Tsalu PV, Tshibangu DST *et al*. Chemical fingerprint and anti-sickling activity of Rosmarinic acid and Methanolic extracts from three Species of *Ocimum* from DR Congo. J of Biosciences and Medicines. 2016; 4:59-68.

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