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Botanical, Physicochemical, and Phytochemical Studies of *Zanthoxylum zanthoxyloides* (Lam.) Zepernick and Timler (Rutaceae) Stem and Root Bark

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Abstract: *Zanthoxylum zanthoxyloides*, a medicinal plant overexploited in Burkina Faso due to the use of its roots for therapeutic purposes has been studied and it seems that the profile of the powder from stem and root bark are almost similar. This study aims to compare the botanical, physicochemical, and phytochemical parameters of *Z. zanthoxyloides* powder from stem and root bark for rational use. Botanical characters were carried out while methanol and water extraction yield, residual moisture, total ash, and insoluble ash in chlorohydric acid were estimated. Phytochemical screening was realized by the HPTLC using a THF-toluene-formic acid-water (59:30:7:4, v /v/v/v) solvent system. The total phenol and flavonoid content of both methanolic and aqueous extracts were quantified and their antioxidant activities were assessed using the ferric ion-reducing power and DPPH methods. Important fibers were observed in the root powder. The total percentage of ash and hydrochloric acid ash in the stem and root barks was almost similar in both samples, while the methanol yield (10.78±1.1%) was significant for the roots. The HPTLC profile of the methanolic extracts exhibited the presence of gallic acid and derivatives of chlorogenic acid. The total phenol content of the methanolic extracts from stem and root barks was significantly different (p-value <0.001) within the three sites. However, no significant difference (p-value >0.05) was observed in the phenol content of the aqueous extracts from stem and root barks. The Ferric reducing capacity of water extracts of the stem (104.69±5.17 mg EAA/g) and root bark (83.56±2.16 mg EAA/g) was higher than methanol extract. DPPH inhibition of methanolic extract of the stem (56.84±0.96%) and root bark (58.38±0.84%) was more important than water extract. These results can contribute to *Z. zanthoxyloides* monography edition, standardization of their raw materials, and his rational use for phytomedicines development.

Keywords: *Zanthoxylum zanthoxyloides*, Botanical Characters, Physicochemical, Phytochemical, Antioxidant Activities, Chromatographic Profile

Introduction

Zanthoxylum zanthoxyloides (Lam.) Zepernick and Timler (*Rutaceae*) present in the western part of Burkina Faso are involved in the treatment of several diseases such as hypertension, diabetes, diarrhea (Ogunbolude *et al*., 2014) malaria (Goodman *et al*., 2019; Okagu *et al*., 2021) sickle cell anemia, labor pain, rheumatisms (Cissé *et al*., 2022) and cancers (Andima *et al*., 2019). The stem bark is used for dysentery, cholera, abdominal and stomach ulcers, and roots for arterial hypertension, parasites, and sickle cell disease (Ouédraogo *et al*., 2020). Moreover, *Z. zanthoxyloides* raw material is involved in the preparation of phytomedicines for sickle cell anemia treatment such as FACA® in Burkina Faso and DREPANOSTAT® in Togo (Villaret *et al*., 2018).

In addition, to identifying strategies for species rational use and protection, a study was conducted on genetic and metabolomic variability of *Z. zanthoxyloides* plant specimens from Burkina Faso (Ouédraogo *et al*., 2019a). Statistical analysis based on infrared spectral profiles comparing plant organs highlighted the phytochemical similarity between stem bark and root powder which differed from leaves (Ouédraogo *et al*., 2020). Therefore, stem bark could substitute roots used in some remedies and medicinal preparations whose effect is associated with phenolic compounds such as vanillic acid and quinic acid di-esters derivatives (Ouattara *et al*., 2009). Indeed, vanillic and hydroxybenzoic acids from *Z. zanthoxyloides* and *Cajanus cajan* (L.) Huth has been identified as responsible for the anti-sickling activity, and the efficacy of these plants in the management of sickle cell disease is well documented (Imaga, 2013). Elsewhere, Ouattara *et al*. (2009) showed that *Z. zanthoxyloides* root bark contained burkinabin a, b, and c which are vanillic acid esters of quinic acid. Thus, burkinabin c had significant anti-sickling activity like sodium cromoglycate (Ouattara *et al*., 2009; Souannavong, 2017).

Furthermore, biological potential and economic purposes of *Z. zanthoxyloides* constitute a source of interest in explaining its overexploitation, particularly its roots which represent the favorite organ of traditional medicine practitioners (El Jemli *et al*., 2016). However, the progressive rarefaction of the species in Burkina Faso is becoming more and more real. Thus, it remains essential to preserve the species *Z. zanthoxyloides* from non-rational overexploitation by identifying standardization criteria based on a specific content of phenolic acids as biomarkers of biological effects, allowing effective substitution of the roots by the bark of the stem. The choice of phenolics as raw material biomarkers is a suitable tool for discriminating plant organs (Mohamed *et al*., 2018).

The present study aims to compare botanical, physicochemical, and phytochemical characteristics of *Z. zanthoxyloides* stem and root bark for monographing, raw material standardization, and species conservation.

Materials and Methods

Materials Description

Z. zanthoxyloides is a thorny tree or shrub with rough, slightly vertically fissured bark that is gray to beige in color (Akhtar *et al*., 2012). Spines are solitary and arranged irregularly on the branch, the twigs under the petioles, and sometimes on the midrib of the leaves. Leaves are alternate, odd-pinnate, glabrous with opposite or alternate leaflets, obovate or elliptical. They give off a lemongrass smell. The inflorescences are loose panicles, terminal or arranged at the base of the leaves, with short and sometimes long branches 5-25 cm long. Flowers are white or greenish in color. Fruits are lobular capsules 5-6 mm in diameter which reveal a black and shiny seed revealing translucent spots.

Plant Material Sampling

Plant material was stem and root barks of *Z. zanthoxyloides* (Fig. 1) collected from 3 sites in Burkina Faso (Niangoloko, Sideradougou, and Orodara). One hundred eight (108) samples were collected of which twelve mature trees were randomly selected from each site from December 2017 to January 2018. At each site, 12 samples of leaf stem and root bark were collected from the 3 sites. The samples were pulverized and the powders (Fig. 1) were packaged in «Ziplock» plastics.

Botanical Characterization

Ocular observations were carried out to assess the color and texture of the powders.

A small quantity of stem/root samples was taken for each sample to form a single batch per organ and per origin for microscopic observations.

Stem and root bark powders have been described from optical microscope observation in the presence of 10% KOH with 10 and 40× objectives on a 5× eyepiece for a magnification of 50-200. Observed microscopic elements were identified by comparison using herbal drugs and herbal drug preparations part of European Pharamacopoeia (2019) and the "Atlas of Microscopy of Medicinal Plants, Culinary Herbals and Species" manual (Jackson and Snowdon, 1990).

Physicochemical Characterization

Determination of Residual Moist Content

Moisture content was estimated using MAC 110 moisture analyzer (RADWAG; Poland) at 120°C until constant weight following the method of Difonzo *et al*. (2022). Approximately 1.0 g of powder was used for each sample and then weighed (n = 3).

Fig. 1: *Zanthoxylum zanthoxyloides* specimen; (A): Leaves; (B) Fruits; (C) Roots

Total Ash and Acid Insoluble Ash

Total ash and acid insoluble ash were estimated following the adapted method of Kadam *et al*. (2013). Total ash total ash was obtained by calcining 5 g of powder in a 600°C oven for 5 h after cooling. The residual total ash content was calculated according to the following formula:

$$Total\ ash\ (\%) = \frac{Average\ mass\ of\ bottom\ ash}{Mass\ of\ the\ test\ socket} \times 100 \quad (1)$$

Acid-insoluble ash was obtained by using the total ashes and boiling it in an Erlenmeyer flask containing 20 mL of hydrochloric (10%) acid for 20 min in a water bath. After cooling, the solution was filtered on ashless filter paper which was then dried in an oven and then calcined for 5 h in the oven at 600°C then weighed after cooling. The percentage of acid insoluble ash (% AIA) was then calculated according to the formula below:

$$Ash\ insoluble\ (\%) = \frac{Mass\ of\ ash\ insoluble\ acid}{ness\ of\ the\ test\ socket} \times 100 \quad (2)$$

Methanol and Aqueous Extraction Yield

Extraction by maceration under continuous stirring for 24 h was carried out on 3 g of powder with 50 mL of methanol following the adapted method of Oroian *et al*. (2020). After filtration, extracts were centrifuged at 3000 rpm and then dried in an oven at 50°C after that weighed and stored (n = 3).

Aqueous extraction was done by maceration under continuous stirring for 24 h at room temperature was carried out on 3 g of powder with 30 mL of distilled water. The extract was filtered on paper and was then freeze-dried to powder. The extraction yield was obtained according to the formula below:

$$Extraction\ yield\ (\%) = \frac{Mass\ of\ dry\ extract}{Mass\ of\ initial\ test\ portion} \times 100 \quad (3)$$

Phenolic Compounds Screening

About 1 g of sample was extracted by methanolic maceration under sonication for 30 min. The preliminary standards of HPTLC were implemented first manually according to the procedure of Reich and Schibli (2007); and Akhtar *et al.* (2012). The following compounds were spotted on a silica gel 60 F254 (Merck) glass HPTLC plate using an automatic TLC sampler (CAMAG): 2 µL of methanolic extracts and references: Myricetin (0.55< Retention factor (Rf)<0.79), isorhamnetin (0.4< Rf <0.5), hyperoside (0.3< Rf <0.4), rutin (0.15< Rf < 0.23), Quercetin (0.7< Rf <0.8) at 1 mg/mL, Gallic acid (0.67< Rf <0.76) at 0.5 mg/mL, chlorogenic acid (0.25< Rf <0.4) at 0.5 mg/mL. Chromatographic development was conducted in an Automatic Developing Chamber (ADC2) with a THF-toluene-formic acid-water (59: 30: 7: 4, v /v/v/v) mobile phase in pre-saturated atmosphere. Derivatization was obtained using CAMAG derivatizer with 2 mL of Natural Products/Polyethylene Glycol (NP/PEG) reagent. The compound was detected and integrated using CAMAG visualizer 2 at 254 and 366 nm and WinCATS software for data collection. The choice of methanolic extracts was considered for the HPTLC study about the demonstration of most of the active principles (which are phenolic compounds) found and better antioxidant activities (Tine *et al*., 2017a).

Total Phenolic and Flavonoid Contents

Total Phenolic Content

The determination of total phenolics was carried out according to the procedure described by Singleton *et al*. (1999). Three readings were taken per extract sample and the results were expressed in mg gallic acid equivalent per 100 mg of dry extract.

Total Flavonoid Content

Total flavonoids were determined using a Dowd method adapted from Arvouet-Grand *et al*. (1995). Three readings were taken per extract sample and the results were expressed in milligrams of quercetin equivalent per 100 mg of dry extract (mg EQ/100 mg).

Antioxidant Activities

Ferric Reducing Antioxydant Power Assay (FRAP)

FRAP assay was determined using the adapted method from Lamien-Meda *et al*. (2008). Plant extract (100 µL, 1 mg/mL in methanol) was mixed with a phosphate buffer (250 µL, 0.2 m, pH 6.6) and with a solution of potassium hexacyanoferrate (250 l, 1% in water). After incubation (30 min, 50), trichloroacetic acid (250, 10% in water) was added and the mixture was centrifuged (3000 rpm for 10 min). The supernatant (125 µL) was mixed with water (125 µL) and a freshly prepared $FeCl_3$ solution (25 µL 0.1% in water) before reading absorbance at 700 nm. Ascorbic acid was used for the standard curve (R^2 = 0.99). Reducing capacity was expressed as milligrams of ascorbic acid equivalent per gram of plant extract (mg EAA/g).

DPPH Assay

Anti-radical activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical as described by Lamien-Meda *et al*. (2008) was used. This method is based on measuring the absorbance at 517 nm when a stable free radical DPPH reacts with an antioxidant. Stock solutions of the extracts (methanolic and aqueous) were prepared by dissolving

them respectively in methanol and distilled water at concentrations of 1 mg/mL. For each extract, three (03) tests were carried out by mixing 100 µL of extracts and 200 µL of DPPH (0.6 mm in ethanol). After 15 min of incubation, the reading was taken at the wavelength of 517 nm against a blank using a spectrophotometer (BIO-TEK Instruments, INC). The control is composed of 100 µL of methanol or distilled water and 200 µL of DPPH.

The percentages of inhibition of DPPH radicals by the extracts were determined according to the following formula:

$$DPPH\ inhibition\ (\%) = \frac{Absorbance\ the\ blank\ sample - Absorbance\ of\ sample}{Absorbance\ of\ sample} \times 100 \qquad (4)$$

Data Analysis

Minitab 19.1 software was used for the analysis of variance. The comparison of the means was made by Tukey's test and was statistically significant for $p<0.05$.

Results

Botanical Characterization

Macroscopic observations indicated that the root and stem bark powders were ochre-colored (Niangoloko and Sideradougou) to asparagus-yellow (Orodara), hard and rough in texture, and spicy in taste (Fig. 2).

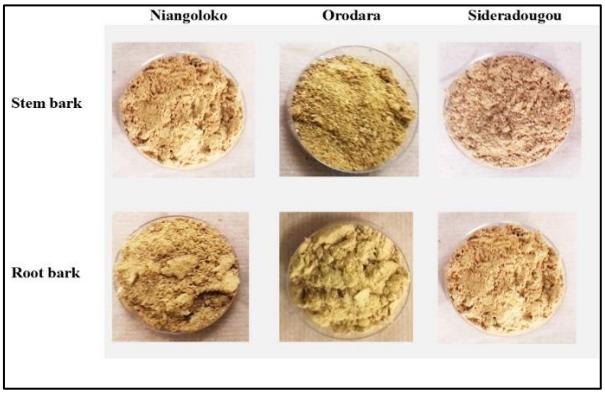

Fig. 2: Samples of powder of *Zanthoxylum zanthoxyloides*

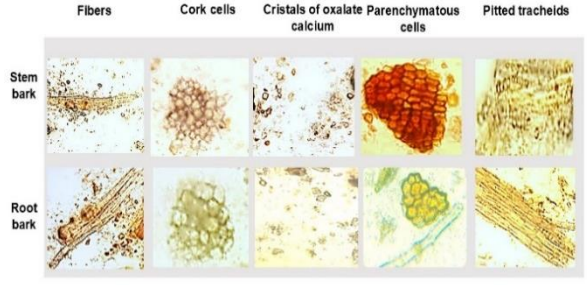

Fig. 3: Microscopic observation of *Zanthoxylum*

Microscopic observation showed that *Z. zanthoxyloides* stem and root bark powder contains fibers, cork cells, parenchymentous cells, pitted tracheid, and calcium oxalate crystals (Fig. 3).

Physicochemical Characterization

Physicochemical parameters for the purity and quality of *Z. zanthoxyloides* stem and root bark powder are presented in Table (1). Residual moisture content average was 7.73±0.50 and 7.96±0.25%, and total ash associated with mineral content was 6.36±0.46 and 6.33±0.46% for stem bark and root bark respectively while insoluble ash in 10% hydrochloric acid content associated to silicious content showed similar values of 2.22±0.57 and 2.11±0.57% for stem and root bark respectively. There was no significant difference between the evaluated parameters of samples from Niangoloko and Sideradougou for total ash, however, those collected in Orodora were significantly lower (p-value <0.001).

The methanol extraction yield was higher in roots (10.78±1.1%) compared to stem bark (4.45±0.32%) which indicated that root bark exhibits more methanol-extracted compounds compared to stem bark. Furthermore, the collection site and significantly the (p-value <0.001) impacted the methanol extraction yields, which varied from 2.70±0.26-6.60±0.3% for stem bark respectively from Niankologo to Sideradougou compared to those of root bark that varied from 8.78±2.52-14.04±0.36% from Orodara to Sideradougou. Sideradougou *Z. zanthoxyloides* specimen contained most of the methanol-extracted compounds. Aqueous extract yield is more important in stem bark (5.25±0.13%) than root bark (4.38±0.16%) and was higher than methanolic extract stem bark yield. Aqueous extract yield stem bark was 6.78±0.24, 5.6±0.07, and 3.38±0.09% in Sideradougou, Niangoloko, and Orodara, respectively. For root bark, aqueous extract yield was more important for Orodara root bark (5.32±0.06%) followed by Sideradougou root bark (4.66±0.1%) and Niangoloko root bark (3.18±0.27%).

Phytochemical Screening

HP-TLC profile of *Z. zanthoxyloides* stem and root bark methanolic extracts from three collection sites indicated the presence of simple phenolic analogs with similar retention factor (included in 0.67< Rf <0.76) and the same blue color to gallic acid standard (at Rf ≈ 0.72 included in 0.67< Rf <0.76). Also, quinic acid phenolic esters derivatives with variate Rf around 0.28 included in (0.25< Rf <0.4), but the same emerald green color to chlorogenic acid (with Rf around 0.36 included in 0.25< Rf <0.4) (Figs. 4-5).

Table 1: Physicochemical parameters of stem and root bark of *Zanthoxylum zanthoxyloides*

Plant part	Site	Residual most Yield (%)	Total ash content (%)	Acid insoluble HCl (%)	MeOH extraction 10% (%)	Aqueous extraction yield (%)
Stem bark	Niangoloko	7.53±0.30^{a}	7.60±0,05^{a}	2.33±0.57^{a}	2.70±0.26^{c}	5.06±0.07^{b}
	Orodara	8.21±0.54^{a}	3.50±0.00^{b}	1.67±0.57^{a}	4.05±0.40^{b}	3.38±0.09^{c}
	Sidéradougou	7.44±0.67^{a}	8.00±0.09^{a}	2.67±0.57^{a}	6.60±0.30^{a}	6.78±0.24^{a}
	Mean	7.73±0.50	6.36±0.46	2.22±0.57	4.45±0.32	5.25±0.13
Root bark	Niangoloko	7.49±0.05^{b}	7.40±0.04^{a}	2.33±0.57^{a}	9.51±0.38^{b}	3.18±0.27^{c}
	Orodara	8.66±0.00^{a}	3.50±0.00^{b}	1.33±0.57^{a}	8.78±2.52^{c}	5.32±0.06^{a}
	Sidéradougou	7.75±0.12^{b}	8.10±0.10^{a}	2.67±0.57^{a}	14.04±0.36^{a}	4.66±0.15^{b}
	Mean	7.96±0.25	6.33±0.73	2.11±0.57	10.78±1.10	4.38±0.16

Results are presented as mean ± SD (n = 3). Values that do not share any letter are significantly different

Table 2: Total phenolic (mg EAG/100 mg of dry extracts) and flavonoid (mg EQ/100 mg of dry extracts) contents of stem and root bark of *Zanthoxylum zanthoxyloides*

		Total phenolic content	Total phenolic content	Total flavonoid content	Total flavonoid content
Plant part	Site	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
Stem bark	Niangoloko	151.9±11.20^{a}	94.16±5.12^{a}	3.40±0.21^{a}	1.90±0.04^{a}
	Orodara	124.37±9.70^{a}	93.11±5.44^{a}	2.98±0.10^{a}	1.61±0.01^{a}
	Sideradougou	46.41±1.53^{b}	115.70±8.35^{a}	2.58±0.07^{a}	1.79±0.01^{a}
	Mean	105.56±7.47	100.99±6.30	2.98±0.13	1.77±0.02
Root bark	Niangoloko	93.57±7.09^{a}	88.18±5.59^{a}	2.90±0.20^{b}	0.85±0.02^{a}
	Orodara	68.87±8.05^{b}	91.86±8.15^{a}	4.85±0.07^{a}	0.17±0.00^{b}
	Sideradougou	50.29±5.79^{c}	91.20±7.23^{a}	1.38±0.03^{b}	0.92±0.02^{a}
	Mean	70.91±6.97	90.40±6.99	3.04±0.10	0.65±0.01

Results are presented as mean ± SD (n = 3). Values that do not share any letter are significantly different

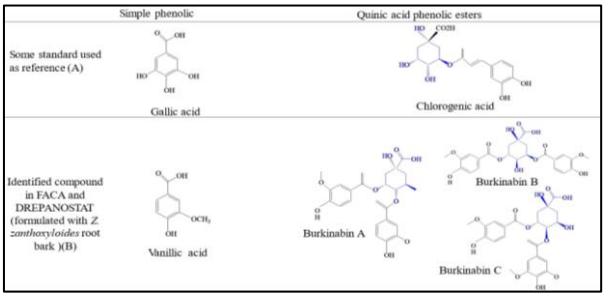

Fig. 4: Chemical structures of phenolic standard references; (A) antisickling compounds isolated from *Z. zanthoxyloides* root bark; (B) (Villaret *et al.*, 2018)

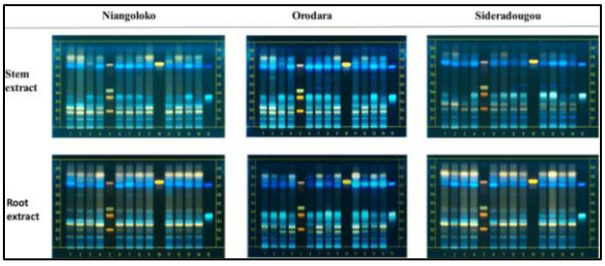

Fig. 5: HP-TLC polyphenol profile of methanolic extract of *Z. zanthoxyloides* stem and root bark, collected in three independent site

Legend:1-4: Targeted organ methanolic extract, 5: Rutin, myricetin, hyperoside, isorhamnetin (0,25 mg/mL) standard references, 6-9: Targeted organ methanolic extract, 10: Quercetin (1 mg/mL) standard reference, 11-14: Targeted organ methanolic extract, 15: Chlorogenic acid, gallic acid (0,5 mg/mL) standard references. Observed under UV at 366 nm after derivatization (NP/PEG 400).

Total Phenolic and Flavonoid Contents

The results revealed significant differences (p-value <0.001) in phenol content between stem and root barks from the studied sites (Table 2). Stem bark exhibited an average value of 105.56±7.47 mg EAG/100 mg of dry matter, while root bark had an average of 70.91±6.97 mg EAG/100 mg. In contrast, there was no significant difference (p-value >0.05) in the total phenol content of the aqueous extracts from stem and root barks with average means of 100.99±6.30 mg EAG/100 mg and 90.40±6.99 mg EAG/100 mg of dry matter in stem and root bark, respectively. For total flavonoids, there was no significant difference (p-value >0.05) for stem bark, but a significant difference was found for root bark (p-value <0.001). The average total flavonoid content of the methanolic extracts from stem bark (2.98±0.13 mg EQ/100 mg of dry matter) and root bark (3.04±0.10 mg EQ/100 mg of dry matter) was almost similar. For the aqueous extracts, no significant difference (p-value >0.05) was observed in stem bark, but a significant difference (p-value <0.001) was found in the root bark with an average total flavonoid content of 1.77±0.02 mg EQ/100 mg of dry matter and 0.65±0.01 mg EQ/100 mg of dry matter in stem and root bark, respectively.

Antioxidant Activities

Ferric-reducing capacity and DPPH inhibition activity are summarized in Table 3.

Table 3: Antioxidant activities

		FRAP (mg EAA/g)	FRAP (mg EAA/g)	DPPH inhibition (%)	DPPH inhibition (%)
Plant part	Site	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract
Stem bark	Niangoloko	79.70±5.95^{a}	143.52±8.56^{a}	54.37±0.91^{b}	55.41±0.40^{a}
	Orodara	81.71±4.49^{a}	47.84±1.63^{c}	60.21±1.05^{a}	57.13±1.54^{a}
	Sideradougou	73.93±3.31^{a}	122.71±5.34^{b}	55.96±0.92^{b}	50.74±0.09^{b}
	Mean	78.45±4.58	104.69±5.17	56.84±0.96	54.42±0.94
Root bark	Niangoloko	57.34±1.26^{a}	60.01±0.36^{b}	59.08±0.31^{a}	46.18±2.09^{a}
	Orodara	44.33±1.71^{b}	119.01±1.73^{a}	57.03±1.82^{a}	39.45±2.08^{ab}
	Sideradougou	38.65±2.05^{c}	71.67±4.41^{b}	59.02±0.40^{a}	37.21±3.61^{b}
	Mean	46.77±1.82	83.56±2.16	58.38±0.84	40.94±2.59

Results are presented as mean ± SD (n = 3). Values that do not share any letter are significantly different

The ferric-reducing capacity of methanolic extracts was higher in stem bark (78.45±4.58 mg EAA/g) compared to root bark (46.77±1.82 mg EAA/g). No significant difference (p-value>0.05) was observed for stem bark samples from collection sites while root bark samples were all different. In fact, Niangoloko root bark value was (57.34±1.26 mg EAA/g) higher compared to Orodara (44.33±1.71 mg EAA/g) and Sideradougou (38.65±2.5 mg EAA/g).

The ferric reduction capacity of aqueous extract was also more important in stem bark (104.69±5.17 mg EAA/g) than in root bark (83.56±2.16 mg EAA/g). Niangoloko stem bark aqueous extract had significant (p-value <0.001) reducing power compared to the other two sites. For root bark aqueous extract, Orodara samples were more significant (p-value <0.01) compared to those of Niangoloko and Sideradougou.

The percentage of DPPH inhibition in methanolic extract for root bark (58.38±0.84) was slightly higher than that of stem bark (56.84±0.96%). For aqueous extract, the DPPH inhibition percentage of stem bark was more important (54.42±0.94%).

Discussion

Z. zanthoxyloides microscopic observations showed that the powder of root bark was richer in fibers than stem bark. Authors also found cork cells, starch, oxalate crystals, *parenchymentous* cells, tracheid, phloem, and xylem in the root bark of *Z. zanthoxyloides* (West African Herbal Pharmacopoeia, 2013) and in other species of *Zanthoxylum* (Alam and Saqib, 2015). This information corroborates illustrated microscopic elements in our study to characterize *Z. zanthoxyloides* raw materials. Naked eye observations revealed that the color of the bark powder of the stem and the root of Orodara were almost different from those of the two sites and this could be explained by the clayey nature of the soils of the said site.

Z. zanthoxyloides stem and root bark powders conformed to standard recommendations for humidity between 10 -20% given by Akhtar *et al*. (2012) to better manage microorganisms' growth (bacteria and fungi). This indicates that our powders have been well-dried for good quality. Elsewhere, residual moisture recorded from *Z. zanthoxyloides* stem and root bark was like West African Pharmacopeia (WAP) which indicated that a residual humidity value of 7% should not be exceeded (West African Herbal Pharmacopoeia, 2013). The insoluble ash ratio is consistent with the WAP value (<3.5%) while total ashes ratios are higher than the WAP value (<5%) for all samples, except root barks from Orodara. These differences in physicochemical parameters could be linked to the geographic origins of *Z. zanthoxyloides* specimens, as littoral states, Togo and Nigeria for WAP versus, Sahelian country, Burkina Faso for this study. Better still, work carried out in Nigeria by Olushola-Siedoks *et al*. (2020) on the physicochemical parameters concerning the moisture content of stem bark powder indicated values of 9.47% that also differ from West African Health Organisation criteria (West African Herbal Pharmacopoeia, 2013).

Elsewhere in previous studies, vanillic acid was reported in *Z. zanthoxyloides* stem and root bark (Ouattara *et al*., 2009; Okagu *et al*., 2021), and their quinic acid esters derivatives such as burkinabins b and c which have been identified as roots active components involved in the production of anti-sickling phytomedicine in Burkina Faso. In fact, analogous chemical structures, gallic acid, and chlorogenic acid were used as HPTLC profiling standard references (Mosić *et al*., 2019). Then, simple phenolic structure analogs to gallic acid were identified and attributed to C6-1 phenolic compounds like vanillic acid and others according to similar Rf and the same blue color of spots (Mosić *et al*., 2019). Quinic acid phenolic esters were identified as a reference to chlorogenic acid, a caffeoyl mono-ester of quinic acid, and attributed to others with similar chemical structures such as phenolic di-ester and poly-ester of quinic acid regarding spots emerald grey color (Sen *et al*., 2023). These results present analogs of simple phenolic compounds as general biomarkers of stem and root barks of *Z. zanthoxyloides*, chlorogenic acid, or phenolic acid mono-ester of quinic acid for stem bark compared to other phenolic acid poly-esters of quinic acid, more specific for

root bark. Yet, the presence of chlorogenic acid in stem bark samples from Nigeria had also been reported in the work of Ogunbolude *et al*. (2014). The phytochemical HP-TLC profile of stem and root bark phenolic compounds was comparable and completed our previous work based on the infrared spectral profile (Ouédraogo *et al*., 2019b) that suggested the possible substitution of *Z. zanthoxyloides* root bark raw material by stem bark.

Regarding polyphenol content, the extraction method (methanolic or aqueous) does not significantly affect the total phenol content. However, on the other hand, the extraction method (methanolic or aqueous) has a significant impact on the total flavonoid content.

The higher phenolic content in the methanolic extracts may be responsible for their higher antioxidant activity. This is supported by the correlation between the antioxidant activity and the total phenolic content observed in other studies (Olajuyigbe and Afolayan, 2011).

Elsewhere, methanolic extract samples ferric reducing power, using ascorbic acid as a reference, also support observations linked to the type of phenolic compounds found in raw materials. *Z. zanthoxyloides* stem bark, with similar phenolic compounds in TLC profile offer a better antioxidant power than roots with different extraction yields. In general, aqueous extract had more reducing power than methanol extract. That said the work of El Jemli *et al*. (2016) showed the antioxidant capacity of leaf aqueous extract of three species in their study about ferric reducing capacity and this comforted our results. DPPH inhibition activity was slightly higher for methanol extract compared to aqueous. Both extracts exhibit the same anti-radical effect. The work of Adekunle *et al*. (2012) on the antioxidant activity of stem bark of *Z. zanthoxyloides* using the DPPH and chelating iron showed significant antioxidant activity which is consistent with our findings. The DPPH inhibition percentages were slightly higher in root bark methanolic extracts compared to stem bark, while aqueous extracts showed higher inhibition in stem bark, which agrees with those reported by Olushola-Siedoks *et al*. (2020).

The antioxidant activities observed in this study are consistent with previous studies on *Z. zanthoxyloides* species, which have shown significant antioxidant and antimicrobial activities (Tine *et al*., 2017b; Goodman *et al*., 2019). The presence of phenolic compounds such as gallic acid in the methanolic extracts of *Z. zanthoxyloides* stem and root bark supports the antioxidant activities observed. The differences in antioxidant activities between collection sites suggest that the plant material from certain sites may be more effective in terms of its antioxidant properties. In addition, the identified compounds in *Z. zanthoxyloides*, particularly vanillic acid and quinic acid esters, have been linked to antisickling properties (Ouattara *et al*. 2009; Villaret *et al*., 2018). These compounds are known to possess antioxidant and anti-inflammatory activities, which contribute to their potential therapeutic benefits (Tine *et al*., 2017c).

The detailed analysis of the plant's properties provides valuable insights into its potential uses and applications, which can inform conservation efforts and the development of new products.

The study highlights the importance of standardizing the collection and processing of *Z. zanthoxyloides* raw materials to ensure consistency in quality and purity. This standardization can help in the conservation of the plant by reducing the impact of unsustainable harvesting practices and promoting sustainable collection methods (Chen *et al*., 2016). The findings on regional variations in physicochemical parameters and antioxidant activities suggest that different collection sites may have distinct characteristics. This knowledge can inform conservation strategies by identifying areas with higher quality and more effective plant material, which can help in the long-term conservation of the species. The phytochemical profiling of *Z. zanthoxyloides* stem and root bark powders provides a comprehensive understanding of the plant's chemical composition. This information can be used to develop targeted conservation strategies, such as protecting specific habitats or ecosystems that support the plant's growth.

The study's results on the antioxidant activities of *Z. zanthoxyloides* extracts support the potential use of the plant in the development of new phytomedicine. The identified compounds, such as vanillic acid and quinic acid esters, have been linked to anti-sickling properties and possess antioxidant and anti-inflammatory activities (Ouattara *et al*., 2009), which can contribute to their therapeutic benefits.

Conclusion

This study highlights the similarities and differences between the stem and root bark of *Zanthoxylum zanthoxyloides*. Botanical and physicochemical parameters show similarities, except for the methanolic extraction yield, which is higher in root bark. The phytochemical and total phenolic analysis reveals that methanolic extracts of stem bark and aqueous extracts exhibit potent antioxidant activity. Phenolic compounds can be used as biomarkers for *Z. zanthoxyloides* raw materials. The study also identifies the presence of chlorogenic acid and phenolic mono-esters of quinic acid in stem bark and other phenolic acid poly-esters of quinic acid in root bark.

The qualitative and quantitative microscopic characteristics of the plant material will be useful in establishing pharmacopoeial standards. The morphology and pharmacognostic aspects of the different parts of the plant, along with phytochemical and physicochemical

studies, will aid in the authentication and quality control of the species. This study contributes to the standardization of *Z. zanthoxyloides* stem and root bark powder for rational use and may inform the monograph edition of the species.

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Author's Contributions

Lassané Ouédraogo: Conceptualized the research project, collected data, analyzed and interpreted the results, drafted the manuscript and critically revised the manuscript.

Aminata Pagnimdebsom Nacoulma: Conceptualized the research project, acquired data, analyzed and interpreted the results, drafted the manuscript and critically revised the manuscript.

Moussa Compaoré: Acquired data, analyzed and interpreted the results, drafted the manuscript, critically revised the manuscript and supervised the research.

Naamwin-So-Bawfu Romaric Meda: Drafted the manuscript and critically revised the manuscript.

Vincent Rouamba: Collected some data, interpreted the results and critical revision of the manuscript.

Martin Kiendrebeogo: Conceived and designed the study, critically revised the manuscript, and supervised the work.

Ethics

This article is original and contains previously unpublished material. The authors confirm that they have all read and approved the manuscript, and that there are no ethical concerns related to this study.

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