

Impact of Domestication on the *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler Root Bark Extracts Chemical Content in Western, Burkina Faso

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Article History: Received 20 July 2025/Accepted in revised form 02 November 2025

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ABSTRACT

Plant domestication is subject to difficulties associated with ecological adaptation and morphological and phytochemical variability that can affect the yield and bioactive composition of the domesticated plant. This study analyses the variability of vanillic acid content in root bark extracts of *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler, comparing wild and domesticated individuals. This analysis was conducted by evaluating plant growth parameters, performing thin-layer chromatography, and determining the presence of phenolic compounds. Thin-layer chromatography analysis confirmed the presence of vanillic acid in all samples. Furthermore, a negative correlation was observed between the stem diameter of the wild individuals and the vanillic acid content ($R^2 = -0.27$; $p = 0.515$) and between the stem diameter of the domesticated individuals and the vanillic acid content ($R^2 = -0.57$; $p = 0.0847$) of the root bark extracts. This suggests that trunk diameter is not a reliable indicator of active compound richness. These results highlight the ecological influence of domestication and plant origin on the secondary metabolism of *Zanthoxylum zanthoxyloides*. The study contributes to a better understanding of intraspecific chemical diversity in relation to domestication, development, and ecological variability of *Zanthoxylum zanthoxyloides*.

Keywords: *Zanthoxylum zanthoxyloides*, Domestication, Vanillic acid, Variability, Ecology

INTRODUCTION

For some time now, the search for substances of natural origin to meet health and social needs has focused particular attention on local plants. These plants represent an immense source of active molecules of plant origin, the richness of which is far from having been fully inventoried. Their therapeutic properties are due to the presence of natural bioactive compounds. These compounds are accumulated in the various organs (leaves, flowers, fruit, stems, roots, bark, etc). This is the particular case of *Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler, also known as *Fagara zanthoxyloides* Lam, is a fairly common species in the thickets and forest galleries of West Africa. In Burkina Faso in particular, *Zanthoxylum zanthoxyloides* is one of the plants of economic, social, and medicinal interest [1,2]. It is an essential component of FACA[®], a highly effective phytomedicine developed through research in Burkina Faso, which relieves sickle cell crises [3,4]. The plant also exhibits anti-inflammatory, antibacterial, anticancer, antimalarial, antihypertensive, insecticidal, and anthelmintic properties [5–7].

However, deforestation, climate change, urbanisation, overexploitation, agriculture, demographic pressure, and bushfires are all factors contributing to the decline of the species' stands and its habitat, endangering the long-term use of the plant [5,8]. Given this observation, it is becoming increasingly essential to domesticate the *Z. zanthoxyloides* species to make the plant resource available and sustainable for people's future needs. However, several major constraints have been reported in numerous studies relating to the domestication of woody resources in general. These include a lack of knowledge about appropriate propagation techniques [9,10], problems associated with adapting plants to a new environment, and issues related to genetic, phytochemical, and morphological variability in cultivated plants that could alter the therapeutic quality of medicinal plants [11–13]. In light of these observations, it is necessary to achieve controlled domestication of the species to ensure its conservation, propagation, and availability to vulnerable populations in Burkina Faso.

The present study is therefore aimed at controlling the domestication of *Z. zanthoxyloides* in Burkina Faso, while ensuring the therapeutic quality of the plant material used to manufacture the phytomedicine. Indeed, previous studies have shown that plant material from Tin (Orodara) had the best active ingredient contents compared to those from Baré and Farako-Bâ [14]. To achieve successful domestication, it is therefore advisable to check phytochemical variability from the natural environment to the controlled environment. To ensure that there is no significant variation between the active principles in the extracts of mother plants from the area of Tin and those in seed-derived plants. The general aim of this study is to assess the impact of domestication effects on the chemical composition of extracts from the root barks of *Z. zanthoxyloides* seedlings from the Tin area. The specific aims were: i) assess the growth performance of domesticated plants; ii) characterise the chemical composition of root bark extracts; iii) assess the influence of plant diameter on vanillic acid content. The hypotheses underlying this study are as follows:

- the qualitative and quantitative chemical composition varies from wild adult plants to domesticated plants.
- stem diameter influences the vanillic acid content of extracts from the root barks of wild and domesticated plants.

MATERIALS AND METHODS

Study Site

The study was conducted in the provinces of Houet and Kenedougou in the Hauts-Bassins region, bordered to the north by the Mouhoun region, to the south by the Cascades region, to the east by the South-West region and to the West by the Republic of Mali. The seeds were collected in the village of Tin, in the commune of Orodara, and the seedlings were produced in a nursery before being planted in Farako-Bâ, in the commune of Bobo-Dioulasso (Figure 1). The province of Houet covers an area of 11,540 km², accounting for 4.21% of the country, with a population of 1,510,638. The province of Kenedougou covers an area of 8307 km² (3.2% of the national territory) with a population of 399,949. The study area is crossed by both the Abidjan-Niamey Road and the Abidjan-Ouagadougou railway line. This favourable geographical position makes it a hub for trade in agricultural products with the rest of Burkina Faso on one hand, and with neighbouring countries on the other.

In this zone, the exploitation of natural resources has been exacerbated by the rapid population explosion, galloping urbanisation, changes in agricultural practices, and the occupation of at-risk areas [15]. The results of specific studies have shown a downward trend in rainfall across all of Burkina Faso's climatic zones, which is expected to become more pronounced by 2050, particularly in the Bobo-Dioulasso area [16]. Gallery forests line the main rivers in the area, with the most common woody species being *Berlinia grandiflora*, *Elaeis guineensis*, *Uapaca togoensis*, and *Cola cordifolia*. The savannahs are characterised by a generally dense and continuous herbaceous carpet, dominated by species such as *Andropogon gayanus*, *A. pseudapricus*, *Schizachyrium sanguineum*, and *Pennisetum pedicellatum*. Its woody stratum includes *Parkia biglobosa*, *Vitellaria paradoxa*, *Khaya senegalensis* and *Terminalia avicennioides*, *Pteleopsis suberosa*, *Hymenocardia acida*, *Cassia sieberiana*, *Daniellia oliveri* and *Parinari curatellifolia* [17].

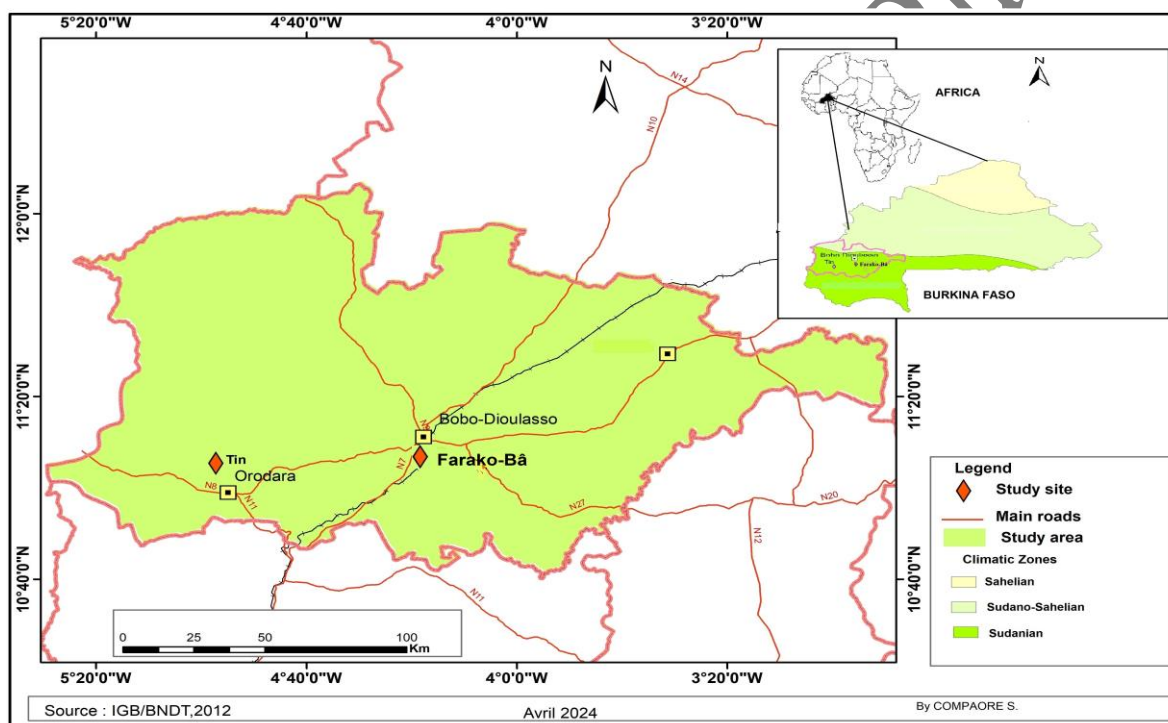


Fig. 1 Location of study area

Data Collection

Evaluation of Growth Parameters

This evaluation was carried out on seedlings from adult individuals considered as mother plants. It concerned growth parameters and parameters relating to external stresses to which the seedlings were subjected. As for growth, this involved dendrometric parameters, such as the height of the seedlings, the diameter at the crown, and the diameters of crown 1 in an east-west direction and crown 2 in a north-south direction. The number of branches (main branches) was also noted per tree measured. For the parameters relating to external attacks, the health of the plants was recorded using a code from 1 to 4, and external signs of visible attacks, such as cuts, pruning, dryness, or withering, were noted.

Collection of Samples

An initial collection was carried out at the village of Tin on adult individuals (diameter at 130cm $\geq$ 5cm) from which seedlings had previously been produced and planted in the controlled environment (Farako-Bâ). Eight (08) adult plants were sampled, bringing the total number of samples to eight (08). The lateral roots were first located and then carefully removed to avoid damaging the plants. During root collection, the circumferences and heights of the individuals were measured.

A second collection was carried out on young plants from adult plants, three years after they had been planted. For this phase, a method destructive to the species was used to collect the root barks [18,19]. Eight (08) *Z. zanthoxyloides* young plants were selected from those of great height to obtain a sufficient quantity of plant material. These plants were then killed in the experimental plot, and the roots were sampled. Their neck diameter and heights were also measured. After each sampling, the roots were carefully washed, and the bark was removed and cut into small pieces. The plant material was then dried and ground according to good practice for harvesting and drying plant material [20].

Preparation of Extracts

5 g of each plant powder was added to 50 mL of solvent (n-hexane, absolute ethanol, or water). The plant powder mixture was homogenised using a vortex mixer and left to macerate for 24 hours at the laboratory's ambient temperature (25°C). After filtration with Whatman filter paper n°1, the extract was centrifuged at 2000 rpm for 10 min with a centrifuger type ALC CENTRIFUGA 4225. The supernatants from each extract were collected for the determination of yield, thin-layer chromatographic fingerprinting, and assays of total phenolics and total flavonoids.

Thin Layer Chromatography

A volume of 5 µL of each extract was deposited on a silica gel G60, F254 stationary phase. The chromatography plate for separating compounds from the n-hexane extract was eluted in a saturated tank containing a mobile phase composed of a 4:1 (v/v) mixture of n-hexane and ethyl acetate. Plates of the absolute ethanol and water extracts from the various samples were eluted in a mobile phase composed of ethyl acetate, methanol, and water in the proportions 7:2:1 (v/v/v). After migration, the chromatograms were observed in daylight and under ultraviolet light at 254 nm and 365 nm.

Determination of Total Phenolic Compounds

Total phenolic compounds were determined using a classic method [21]. The reaction mixture consisted of 1 mL of extract, 1 mg/mL 2N FCR, and 3 mL of 20% sodium carbonate solution. It was left to stand at room temperature for 40 min, and then the absorbance was measured at 760 nm using a spectrophotometer. In the white control tube, the extract was replaced by distilled water. A standard curve ($y = 22.787x - 0.0067$; $R^2 = 0.9994$) was plotted using vanillic acid (1.5 µg/mL), identified as a chemical tracer [14]. Dilution was performed serially from 10^{-1} to 10^{-3} from the initial concentration for the standard curve. The tests were carried out in triplicate. The formula gave the total phenolic concentration of the extract:

$$T_{PT} = \frac{C_{Tube}}{C_i} \times D$$

TPT = Total phenolic content of the extract expressed as Vanillic acid equivalent (EAV)/g
CTube = concentration in mg EAV/mL in the assay tube.

Data Analysed

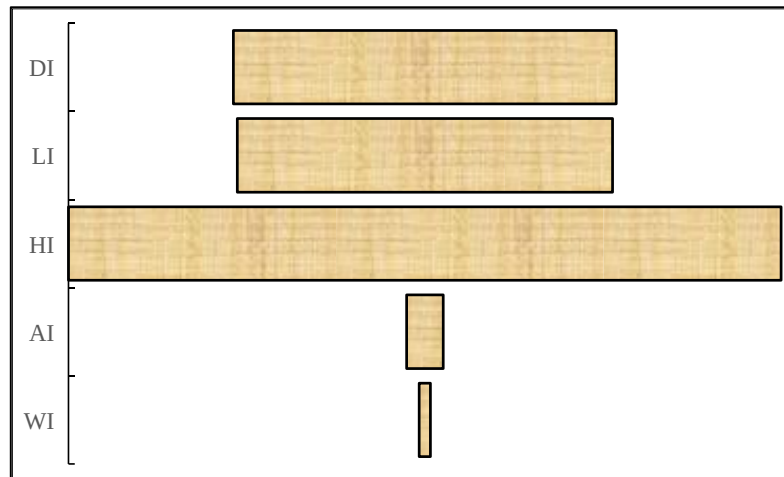
All the data collected was organised in an Excel table. To check whether there was a correlation between the diameters of the plants and the active ingredient content of their extract, on the one hand, and a correlation between the diameters of the plants and the masses of the powders, on the other, we calculated the Pearson correlation coefficient using SPSS 2.0 software. A coefficient close to 1 indicates a strong positive correlation, a coefficient close to -1 indicates a strong negative correlation, and a coefficient close to 0 indicates no significant correlation. A predictive model of bark powder mass as a function of plant diameter was estimated using linear regression with Python 3.11 software. To this end, the coefficient of determination R^2 was determined to understand the extent to which plant diameter (independent variable) explains the variability in the mass (dependent variable) of plant bark powder. To assess the demographic trend of the populations, the individuals were divided into six (06) classes of 0.5 cm diameter and five (05) classes of 20 cm height.

The results of the determination of total phenolic contents were expressed as mean $\pm$ SEM (Standard error of the mean). The results were compared using One-way analysis of variance (ANOVA) with GraphPad Prism version 6.0. A significant difference was considered for $p < 0.05$.

RESULTS

Assessment of the Health Status of Seedlings

Careful observation of *Z. zanthoxyloides* seedlings planted in the plot revealed the presence of both dead plants and plants that had survived three years after planting. Of a total of 475 surviving *Z. zanthoxyloides* plants assessed for health, the majority (93.74%) were healthy, 4.8% were damaged, and 1.46% were wilted (Figure 2). The deaths of individuals were caused by termites, which sometimes left traces, but more significantly by bushfires that occurred during the first year of planting. The attacks were the work of humans, who cut down the aerial parts of the trees uncontrollably. Other individuals, on the other hand, withered as a result of water stress.



DI: Dead individuals, LI: Living individuals, HI: Healthy individuals, AI: Aggressed individuals, WI: Withered individuals

Fig. 2 Health status of *Z. zanthoxyloides* seedlings

Assessment of Population Growth Parameters and Demographic Trends

The growth of *Z. zanthoxyloides* seedlings was normal, and their growth habit varied. The most excellent height recorded was 98 cm, and the most significant number of branches was 5, presented by 5 individuals. The most spreading crowns (H) were 90 cm for H₁ and 96 cm for H₂. The largest diameter at the crown was 4 cm. Figure 3a shows the distribution of the number of individuals by diameter class. This distribution shows a high concentration of individuals in the [0.5-1] and [1-1.5] classes, which account for 73.26% of the total, suggesting an expanding population.

Figure 3b shows the distribution of seedlings by height class. This graph illustrates the highly heterogeneous growth of the plants after three years on the ground. There was a gradual decrease in the number of seedlings as height increased. The majority of seedlings (75.58%) are concentrated in the [0-20] and [20-40] cm classes. This result indicates that the majority of seedlings did not reach a great height within three years of planting. Only a few seedlings in the higher classes managed to grow significantly. This distribution suggests that environmental conditions limited the growth of a large proportion of the seedlings.

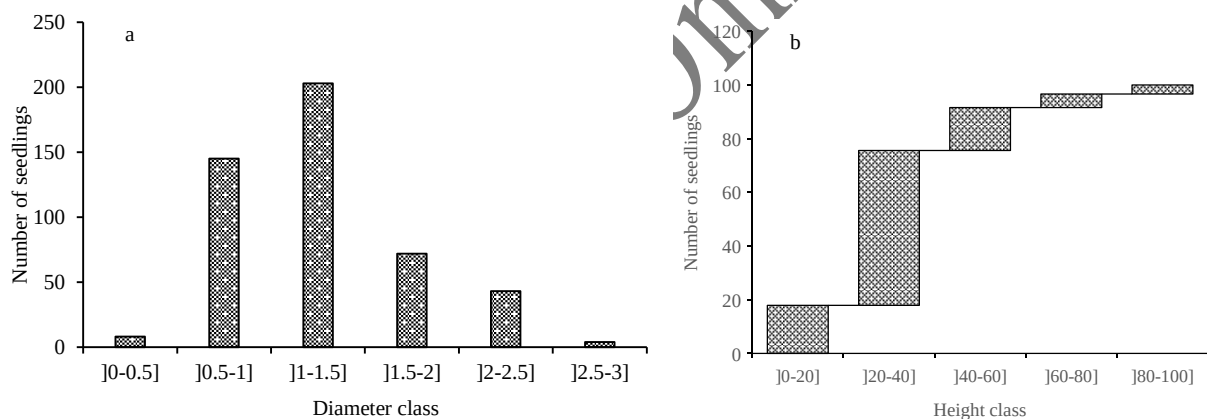


Fig. 3 Distribution of *Z. zanthoxyloides* seedlings by diameter class (a) and height class (b)

Quantity of Root Bark Powder and Extraction Yield of *Z. zanthoxyloides* Seedlings

Analysis of the relationship between the diameter of *Z. zanthoxyloides* seedlings and the mass of root bark powder obtained revealed a moderate positive correlation. The linear regression model (Mass of powder (g) = 16 x Diameter - 3.59 with $R^2 = 0.45$) shows that 45% of the variation observed in the mass of powder is explained by the variation in the diameter at the crown of the plants (Figure 4). This trend suggests that individuals with a larger diameter are generally associated with higher plant matter production. This relationship is illustrated by the sample with the largest diameter (3.82 cm), which also provided the largest mass of powder (74.5 g), thus confirming the direct influence of plant size on powder yield. This result suggests that trunk diameter can be partially used as an indicator of potential raw material yield when selecting plants for domestication or exploitation.

Unlike diameter, seedling height explains no variation in powder mass (Powder mass (g) = -0.0046 x Height (cm) + 41.67 with $R^2 = 0.0001$). This suggests that vertical morphology has no impact on the exploitable mass of the roots.

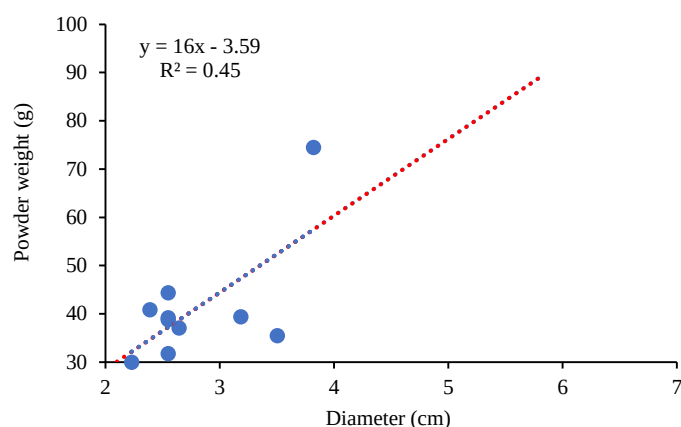


Fig. 4 Plot of the correlation between diameter and powder weight

Table 1 shows the extraction yields of *Z. zanthoxyloides* root bark powders using three types of solvents (hexane, ethanol, and water). Yields varied according to the type of solvent used. The aqueous extract showed higher yields of up to 11.79% (I3), indicating that most of the compounds soluble in the seedling are hydrophilic. The ethanolic extract showed moderate yields. Ethanol, which is effective in total extracts, was used to extract a good quantity of compounds with intermediate polarity (flavonoids, alkaloids, etc.). The hexanolic extract is the least effective, with low yields.

Table 1 Extraction yield according to solvent type

Extraction yield	Hexanolic extract	Ethanolic extract	Aqueous extract
I1	2.86	3.44	7.13
I2	2.88	7.46	11.34
I3	2.87	4.80	11.79
I4	2.33	4.36	6.58
I5	2.04	4.96	7.09
I6	2.14	4.07	5.26
I7	2.03	4.83	5.21
I8	1.82	5.39	7.54

I: individual

Qualitative and Quantitative Chemical Composition of *Z. zanthoxyloides* Root Bark Extracts.

Figure 5 shows the thin layer chromatography (TLC) profiles obtained from ethanolic extracts of *Zanthoxylum zanthoxyloides* root barks, observed under UV light. Visible fluorescent bands (yellow, blue, green) indicate the presence of secondary compounds such as flavonoids, phenols, or alkaloids. The profiles exhibit a high degree of similarity among individuals within the same group, with greater chemical diversity observed in adult plants (a), as indicated by a higher number of bands and more pronounced intensity. In contrast, seedlings (b) showed simpler and less intense profiles, suggesting partial biosynthesis of secondary metabolites at this stage of development. An intense yellow band, well defined and common to all extracts, in both adults (a) and seedlings (b), is visible at the same level of migration on both plates. This band, relatively low on the plate, is characteristic of a moderately polar phenolic compound, compatible with the expected migration of vanillic acid in an ethanolic solvent system. On the plate (a) of adult plants, the band corresponding to vanillic acid is strongly marked, indicating a higher concentration of the tracer in these extracts. On plate (b) from seedlings, although the same band is visible, it is less intense, suggesting a lower vanillic acid content in young plants, linked to incomplete biosynthesis or developmental regulation of phenolic metabolism.

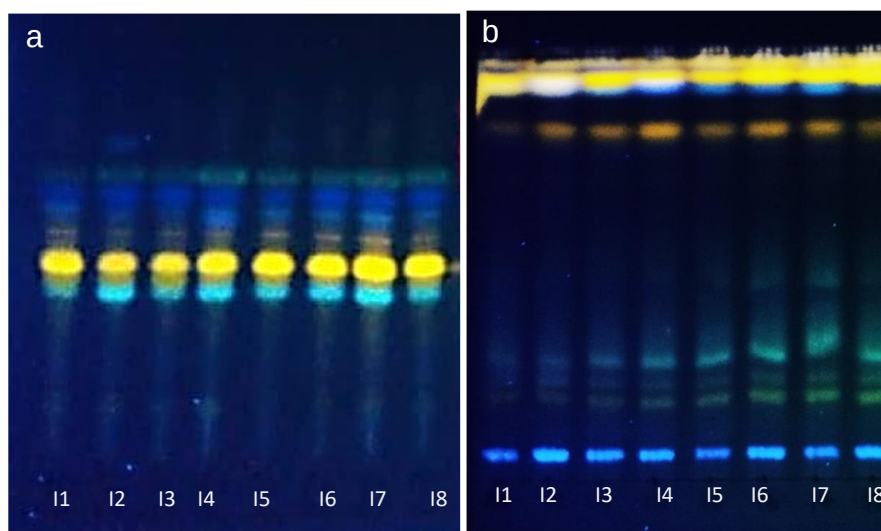


Fig. 5 Thin layer chromatography profiles of ethanol extracts from adult plant roots (a) and seedlings (b)

Figure 6 shows the thin layer chromatography (TLC) profiles obtained from hexane extracts of root bark from adult plants and seedlings of *Zanthoxylum zanthoxyloides*, observed under UV light. In adults (Figure 6a), an intense yellow band with little migration is visible at the top of each layer, indicating the presence of weakly polar compounds, probably lipids, triterpenes, or sterols extracted efficiently with hexane. The size and intensity of this yellow band vary slightly among individuals, suggesting moderate heterogeneity in the concentration of these metabolites. Underneath, a few bluish and greenish fluorescent bands are visible, indicating the presence of less abundant and more polar secondary compounds.

In seedlings (Figure 6b), a constant yellow band is also observed on all layers, similar to those of adult plants. However, the seedlings exhibit a greater diversity of intermediate and lower bands, particularly well-separated greenish and bluish bands. This complex profile suggests greater metabolic diversity at this young stage, perhaps due to more dynamic biosynthetic activity or different regulation of metabolic pathways.

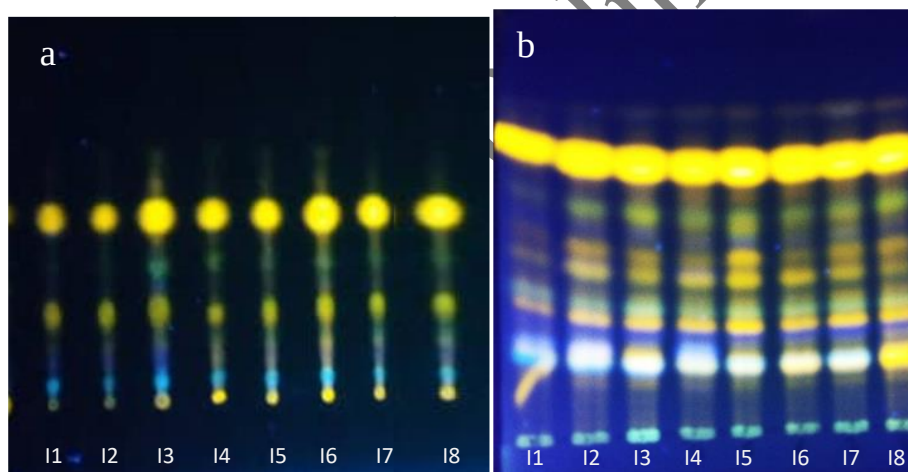


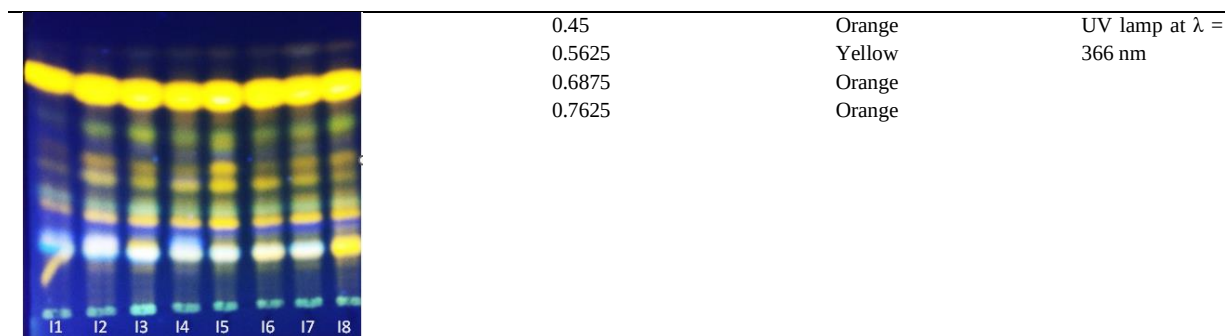
Fig. 6 Thin layer chromatography profiles of hexane extracts from adult plant roots (a) and seedlings (b)

Fingerprint of Domesticated Plants of *Zanthoxylum zanthoxyloides*

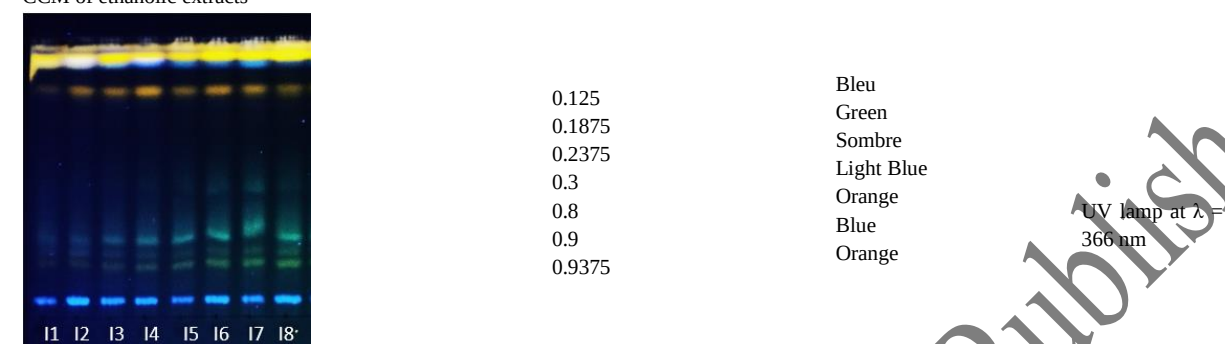
Table 2 presents the characteristics (Rf values and colorations) of the significant spots observed on the chromatograms. The Rf values indicate the mobility of substances on the chromatography plate based on their interaction with the stationary phase and the mobile phase. In total, eight (8) leading bands were observed under ultraviolet light at a wavelength of 366 nm for the hexanic fractions and seven (7) bands for the ethanolic fractions. The Rf values range from 0.125 to 0.9375 in the ethanolic extracts, and from 0.1625 to 0.7625 for the hexanic extracts. These values characterise a diversity in the polarity of the compounds present in each extract. Higher Rf values, such as 0.8 and 0.9, indicate more lipophilic compounds, while lower values, such as 0.125 and 0.1625, suggest more polar compounds. The orange and yellow hues observed in the hexanic extracts may indicate the presence of flavonoids or carotenoids, which are often responsible for these colors. The presence of blue in some fractions may suggest the presence of phenolic compounds. The green coloration would indicate the presence of flavonoids in the extracts [22].

Table 2 RF values of the significant spots of the fingerprint and their coloration

Nature of the extract	RF	Color	Revelation
CCM of hexanic extracts	0.1625	Orange	
	0.2625	Orange	
	0.3125	yellow green	
	0.3875	Orange	



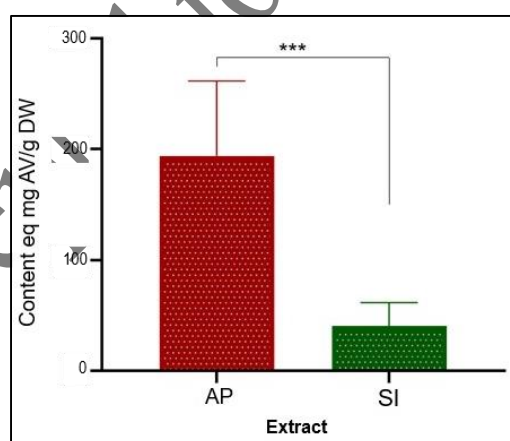
CCM of ethanolic extracts



Influence of Diameter on the Vanillic acid Content of Extracts from *Z. zanthoxyloides* Individuals.

The results in Figure 7 show a significant difference in vanillic acid (VA) content between extracts from adult plants grown in the wild and extracts from domesticated *Zanthoxylum zanthoxyloides* seedlings. The average vanillic acid content in extracts from adult plants was approximately 4.78 times higher than that measured in extracts from seedlings. Mature tissues appear to have a more advanced biosynthetic capacity.

On the other hand, the statistical analyses carried out within each group revealed no significant correlation between the diameter of the individuals and their VA content. The Pearson correlation coefficients obtained are -0.27 for adult plants and -0.57 for seedlings, indicating a non-significant negative trend for both wild plants ($p = 0.515$) and domesticated plants ($p = 0.0847$) (Table 3). In other words, a large diameter is not necessarily associated with a high (or low) vanillic acid content. These results suggest that the content of bioactive compounds is more closely associated with the origin (wild or domesticated) than with the apparent diameter of *Z. zanthoxyloides* individuals.



AP: Adult Plant, SI: Seedlings; DW: dry weight

Fig. 7 Vanillic acid content of extracts from different types of *Z. zanthoxyloides* individuals

Table 3 Pearson correlation between plant diameter and Vanillic Acid content

Variables	Means	Pearson's r	P values
Adult plants			
Diameter	13.906	-0.272	0.515
Content eq mg AV/g DW	194.136		
Seedlings			
Diameter	2.80	-0.57	0.0847
Content eq mg AV/g DW	40.59		

eq: equivalent; AV: vanillic acid; DW: dry weight

DISCUSSION

Growth and Vitality of *Z. zanthoxyloides* Seedlings

Three years after planting, *Z. zanthoxyloides* seedlings showed an overall satisfactory survival capacity. These results confirm the good initial adaptability of the species to the domestication context, in line with the observations made by Akinyele [23], who emphasises the resilience of particular African woody species to controlled cultivation conditions.

On the other hand, the losses recorded are due to significant abiotic and anthropogenic disturbances, such as bushfires, which are frequent in Sudano-Sahelian zones, and termite attacks, which have been identified as factors affecting the physiology of seedlings [24,25]. The majority of seedlings planted are still in the small diameter classes, while a small number are reaching larger diameters.

The growth of the seedlings was probably strongly influenced by the variability of water resources. Indeed, the absence of watering meant that the plants had to rely solely on natural rainfall and the moisture in the soil. Plants that were unable to absorb sufficient water had their growth limited by water stress. Water stress, caused by irregular rainfall, has a significant impact on plant growth and development by affecting essential physiological and biochemical processes, as well as modifying organ morphology and structure [26,27].

Influence of Ecological Parameters on the Quantity of Bark Powder

The study revealed a moderate positive correlation between the diameter of *Z. zanthoxyloides* seedlings and the mass of root bark powder obtained, suggesting that trunk diameter is a partially predictive factor for the powder yield of *Z. zanthoxyloides* seedlings. Authors have reported that the quantity of root bark can be influenced by several factors, including tree species, genetic factors, environmental conditions, and management practices [28–30]. Indeed, in forest ecosystems, positive correlations have been established between various plant species, indicating that trunk diameter at breast height (DBH) is a reliable indicator of coarse root biomass [31,32]. However, the lack of a link between seedling height and powder mass suggests that vertical morphology primarily reflects competition for light and above-ground biomass, without a direct connection to root development [33,34]. These results guide the optimisation of the selection criteria for *Z. zanthoxyloides* plants intended for farming, by including not only morphological parameters such as collar diameter but also in-depth analyses of root structure and chemical composition.

Metabolic Variability Related to the Domestication of *Z. zanthoxyloides*

The results of the thin-layer chromatographic profiles showed the presence of the desired tracer, vanillic acid, both in the extracts from the root barks of adult plants and in those from the root barks of *Z. zanthoxyloides* seedlings. The variation in R_f values for hexanolic and ethanolic extracts of seedling root barks indicates the diversity in terms of polarity of the compounds present. This result was consistent with those obtained during a previous standardisation study carried out using wild plant extracts, showing its interest for future analyses [14].

The assay results showed a marked difference in vanillic acid content between the two types of *Z. zanthoxyloides* plants. On the other hand, the study of the relationship between individual diameter and vanillic acid content revealed a negative correlation in all plant types, suggesting that diameter is not a reliable indicator of vanillic acid content. Indeed, studies have reported that vanillic acid biosynthesis is regulated more by the age of the plant and its origin (wild or domesticated) than by its morphological characteristics [35,36]. The geographical origin of these plants may well lead to significant variations in metabolite levels. Indeed, adult plants are considered wild because they originate from the natural environment and produce a wide diversity of secondary metabolites due to their adaptation to diverse and fluctuating environmental conditions [37,38]. Although these secondary metabolites are also essential for plant survival, their production in domesticated plants may be reduced due to their environmental specificity. From wild to domesticated plants, secondary metabolism varies considerably, influencing their ecological role [39,40].

Additionally, domestication practices result in a reduction in the secondary metabolite content of plants compared to their wild progenitors. This leads to a reduction in secondary metabolites, such as alkaloids, terpenes, and phenolic compounds, which are essential for plant defence and human health [41,42]. This highlights the need to reconcile plant production with maintaining the therapeutic quality of compounds of interest in plant domestication.

CONCLUSION

The study's results revealed the presence of vanillic acid in root bark extracts from all wild and domesticated plants. Its content was higher in wild adult plants, suggesting that physiological maturity and original ecological conditions strongly influence the accumulation of secondary metabolites. Furthermore, a negative correlation was observed between stem diameter and vanillic acid content in both adult and domesticated plants. This shows that diameter is not a reliable indicator of vanillic acid content. The results highlight the importance of conserving wild populations of *Zanthoxylum zanthoxyloides* and integrating ecological factors into cultivation strategies. These parameters must be considered to ensure the sustainable and scientifically sound use of *Zanthoxylum zanthoxyloides*, particularly in light of the growing exploitation of plant resources.

Funding

This research was funded by the National Fund for Research and Innovation for Development (FONRID) under the number FONRID AAP6-12

Acknowledgments

The authors would like to thank all partners, particularly the National Forest Seed Centre (CNSF) and the Institute for the Environment and Agricultural Research (INERA), for their support in carrying out this study.

Conflicts of Interest

The authors have not declared any conflict of interests.

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Accepted to Online Publish