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Full Length Research Paper

Stem bark of *Zanthoxylum zanthoxyloïdes* a possible substitute of root bark for the conservation of the species in Burkina Faso

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***Zanthoxylum zanthoxyloïdes* (Lam.) is used for some health purposes in Burkina Faso. However, human action threatened this species in its native environment and its regeneration is difficult by the way of population. The main focus of this study is to discriminate samples of *zanthoxyloïdes* from different plant populations in order to find good specimens for traditional medicine and thus contribute to the conservation of this species. Plant materials were collected from three study sites named Niangoloko, Orodara and Sidéradougou. Samples powder, mixed with potassium bromide was used for the Fourier-Transform Infrared Spectrometry (FTIR) analysis. Multivariate data analysis was performed to highlight differences in the spectral profile among plant organs. Then, vanillic acid characteristic signals in infrared were identified by using literature data. Results showed that leaf and stem bark spectra were significantly different ($p < 0.001$ and $p < 0.05$, respectively) among the study sites, while root bark spectra were almost identical ($p = 0.19$). Root bark and stem bark both indicated similar patterns under vanillic acid characteristic signals. The use of stem bark instead of roots can be a substitute for root to the sustainable management of this species in its native environment.**

Key words: *Zanthoxylum zanthoxyloïdes*, attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy, multivariate data analysis, vanillic acid.

INTRODUCTION

Zanthoxylum zanthoxyloïdes (Lam.) Zepern. and Timler (Rutaceae) is a West African plant species found in Burkina Faso, with limited geographic distribution, but mostly located in the western part of the country (Eyog Matig et al., 2006; Schmelzer and Gurib-Fakim, 2013).

In traditional medicine, this plant has shown its usefulness (Ynalvez et al., 2012) and well contributed to the health of the population. As an example, this plant is included in phytomedicines (FACA®, DREPANOSAT®) and is used for the health management of the sickle cell

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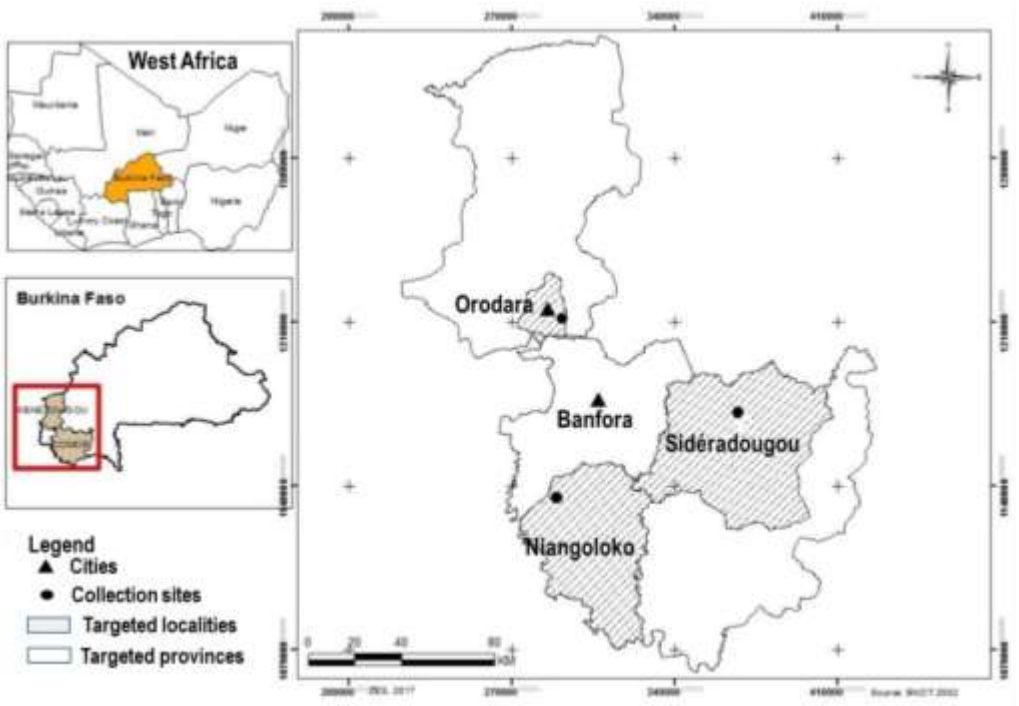

Figure 1. Samples sites.

disease (Imaga, 2010). Besides, ethnopharmacological investigations have been achieved on this plant and several active compounds such as vanillic acid and hydroxy-2-methyl-benzoic acid and derivatives were isolated from the leaf, stems bark and roots bark extracts (Nanfack et al., 2013; Ouattara et al., 2004; Guendéhou et al., 2018). These compounds play an important role in sickle cell disease treatment (Ouattara et al., 2009).

In another side, this plant is threatened and endangered in its native environment (Etsè et al., 2011; Thiombiano and Kampmann, 2010; Nikiema et al., 2010; Adomou et al., 2007*;* Compaoré et al., 2018). The root bark is the most valuable organs used by local populations, traditional healers and local pharmaceutical firms. This is the most important threat to the survival of this species in ecosystems (Diatta et al., 2014). The non-controlled harvesting, the massive destruction of plants by uprooting, and the lack of regeneration, reduced the population of *Z. zanthoxyloïdes* in its natural environment (Etsè et al., 2011).

Hence, it seems important to take initiatives for the sustainable use of this species. For this, spectroscopic techniques can be used to classify samples of any type due to its speed and simplicity, and its low-cost approach (Qi et al., 2017). For example, Fourier transform mid-infrared (FT-MIR) spectroscopy is an effective tool which can scan the chemical composition of a sample in the mid-infrared region (4000 to 400 cm^{-1}) and the fingerprint region created by this technique can give information on the sample's certainty quality (Grunert et al., 2016; Muhtar et al., 2016). In spectroscopy study, Principal Component Analysis (PCA) is the most commonly used technique for the discrimination of the samples (Kamil et al., 2015). PCA is used to show the distribution of the metabolites according to their wavenumbers. The samples with the similar scores are in the similar position while those of dissimilar scores are some distance away (Kamil et al., 2015).

This study aims to discriminate samples of *Z. zanthoxyloïdes* from three sites of Burkina Faso for the selection of better specimen for conservation (regeneration, propagation, cultivation, etc).

For this purpose, this study consisted of a clustering plant samples of *Z. zanthoxyloïdes* collected from three sites of Burkina Faso, and (ii) a determination of the spectral part that discriminates clusters. (iii) In addition, literature data of vanillic acid signals were estimated in the plant parts, to know the distribution of these useful compounds.

MATERIALS AND METHODS

Sites and sampling

Samples were collected in western regions, Niangoloko (N), Sidéradougou (S) and Orodora (O). One hundred and eight (108) samples were collected from twelve (12) randomly selected mature trees per site in fours defined plot (20 m × 250 m). The study sites are as shown in Figure 1. The annual precipitation of the three sites varied from 1000 to 1176 mm. The type of soil was fine particles, eroded and clay soil in Niangoloko, Siéderadougou and

Table 1. Vanillic acid wavenumber and intensity in infrared study.

Active principle	Wavenumber (Intensity)
Vanillic acid	3486 (33); 3099 (58); 2956 (10); 2924 (4); 2854 (11); 2849 (82); 1683 (17); 1598 (19); 1524 (20); 1465 (37); 1466 (33); 1435 (15); 1378 (37); 1299 (16) 1293 (19); 1282 (18); 1239 (20); 1206 (20); 1113 (46); 1030 (35); 919 (62); 883 (62); 820 (68); 807 (65); 768 (49); 766 (46); 722 (74); 689 (72); 637 (66); 611 (66); 583 (77); 542 (70)

Orodara, respectively.

Stem bark samples were taken off with a cutter (30 cm length, 7 mm width) from the median part of the trunk. Leaf and root bark have been collected manually and by using a scissor and a hoe, respectively. All samples were achieved by applying good agricultural and collection practice (WHO, 2004).

Sample preparation

Specimen of tree samples from Niangoloko, Orodara and Sidéradougou were collected and deposited, respectively under the voucher references 3061, 3062 and 3063, in the herbarium of Natural History Laboratory of National Center for Scientific and Technology Research (CNRST). Then, all samples were dried for 2 weeks in a drying oven at 30 to 35°C. The samples were powdered using a mixer (blender SQBL-100, China) to obtain fine particles with homogenate size (≤ 20 mm) according to a method previously described (Rana et al., 2008). Powder samples were stored in a desiccator over dry silica gel until it was used (n=3×36 independent samples).

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectral recording and multivariate data analysis

ATR-FTIR spectra were obtained using NICOLET™ iS™ 5 spectrometer (USA) in the range from 600 to 4000 cm^{-1}. 10 mg of each sample was used to make pellets (10 mm) for spectral recording. Each spectrum was obtained in triplicates (n=3). SIMCA P+ v (12, Sweden) software was used for PCA according to previous methods (Nacoulma et al., 2013; Rana et al., 2008).

The region (1800 to 600 cm^{-1}) and the characteristic signals of one active principle vanillic acid (AIST, 1999) shown in Table 1 were used to cluster samples in a PCA.

RESULTS AND DISCUSSION

Spectra visual observation

The main characteristics of spectra are as shown in Figure 2. The leaf spectra profile from Orodara was different to Niangoloko and Sidéradougou in the range between 3800 and 3400 cm^{-1}, 2400 and 2200 cm^{-1} and 1600 and 1500 cm^{-1}. The stem bark spectra profile from Orodara were very close to the one observed in Niangoloko except in the spectral region between 3800 and 3400 cm^{-1}, while in stems bark samples from Sidéradougou, spectra were different in the range between 3800 and 3400 cm^{-1}, 2400 and 2200 cm^{-1} and 1600 and 600 cm^{-1}.

The spectra profile from roots for the three sites was almost similar except in the region 3800 to 3400 cm^{-1}.

As observed*, Z. zanthoxyloïdes* organs spectra profiles exhibit three characteristics zones: 3800-3400 cm^{-1}, 2300 cm^{-1} and 1600-600 cm^{-1}. The region 3800-3400 cm^{-1} corresponds to OH bending band (Cebi et al., 2019). The region 2300 cm^{-1} indicates alkyne and nitrile region (Suzuki et al., 2005). According to previous studies, the region around 1650 and 1100 cm^{-1} corresponding to a fingerprint region indicates the major cell wall components, such as cellulose, a polyosidic compound, and lignin, a polyphenolic compound (Rana et al., 2008).

According to the spectra pattern, there was a difference within the leaf and stem bark between 1800 and 600 cm^{-1} but root bark spectra were similar within the various provenances. A possible explanation for this is that the distribution of the metabolites in the plants parts is affected by climate and soil (Sampaio et al., 2016).

PCA in the region 1800-600 cm^{-1}

For further information about the variability among samples, PCA was performed on the corresponding first derivative FTIR spectra in the 1800 to 600 cm^{-1} region (Figure 3). Of particular interest was the fingerprint region which includes the absorbed O-H vibration, C-H deformation in lignin and carbohydrates, C-H deformation in cellulose and hemicellulose, C-O stretch in lignin, C-O-C vibration in cellulose and hemicellulose and C-O stretch in cellulose and hemicellulose (Gupta et al., 2015).

The score plot of the first two principal components explained 62.24% of the variability in the dataset (PC1: 39.45% and PC2: 22.79%) for leaves (Figure 3a). Concerning stem bark, PC1: 40.88% and PC2: 30.38% contributed to 71.76% of the spectra variability (Figure 3b). For root bark, the first two principal components (PCs), together accounted for 78.42% of the total variability present in the spectra (Figure 3c).

However, almost all Orodora root bark samples were located on the positive side of PC1. Along the PC2 axis, we observed that all the stem bark samples from Orodara were on the negative side of PC2, while the signals from stem barks from Niangoloko and Sidéradougou are found on both sides of PC2. All the root samples from

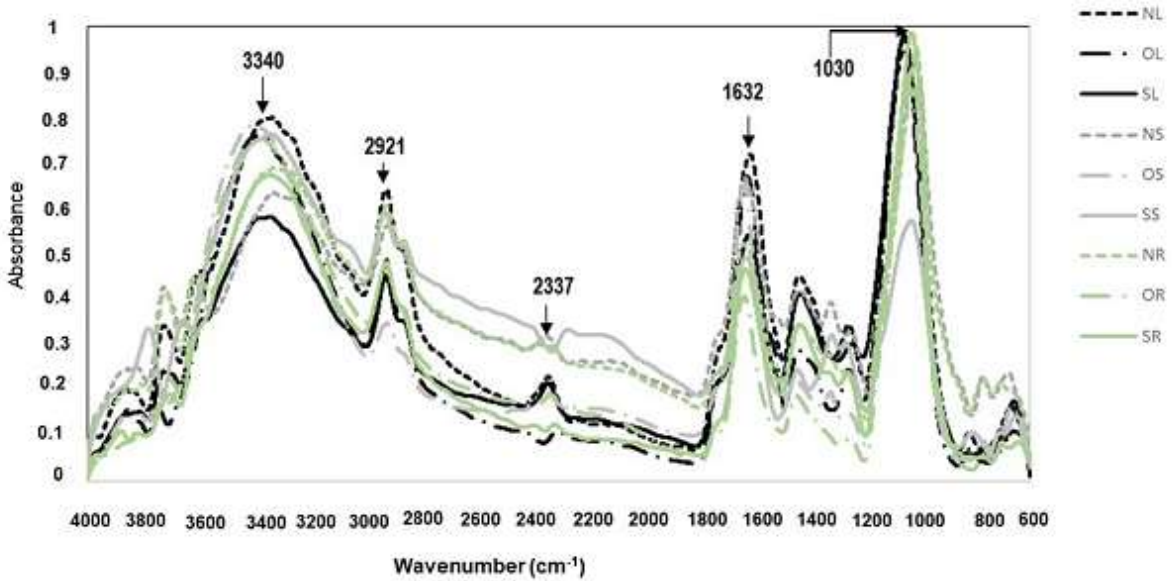

Figure 2. FTIR spectra of *Z. zanthoxyloïdes* leaf, stem bark and root bark in the range from 4000 to 600 cm^{-1} and fingerprint region, respectively. Each spectrum is a mean of spectra from 12 individual trees sampled at Niangoloko (N), Sidéradougou (S), Orodara (O). L corresponds to Leaf, S for Stem bark and R for root bark.

Niangoloko were located on the positive PC2 axis. The root samples from Sidéradougou and Orodara were found to be on the negative side of PC2.

Regarding PC1 and PC2 axes, stem bark from Orodara was distinct to stem bark from Niangoloko and Sidéradougou. These results indicate that there are two groups of metabolites in stem bark. The samples from Orodara were in most cases narrowly grouped together in contrast to those derived from Niangoloko and Sidéradougou. One peculiarity of the Orodora site was the presence of dense clay soil whereas at both other sites soils with loosely packed particles dominated.

The PCA scores based on infrared signals in the range of 1800 to 600 cm^{-1} were tested by a one-way-analysis of variance (ANOVA) to assess spectra variability (Table 2). A difference between leaf spectra and locations could be detected with high reliability ($p = 2.59\times10^{-6} < 0.001$) and as well as a difference within stem bark from *Z. zanthoxyloïdes* metabolites' part profiles across collection sites ($p = 0.01 < 0.05$). No difference for root bark was observed ($p = 0.19$). These results confirm our visual observation of the difference among leaf and stem bark in the range between 1800 and 600 cm^{-1} and resemblance among root barks across the various sites.

This variability of the spectra within the same plant organ can be further interpreted by inspecting the loadings corresponding to PC1 (Figure 4 and Table 3) to identify the signal. An examination of the loadings plot revealed that the variance along the first principal component was mainly driven by signals in the regions 1500 and 1233 cm^{-1} for leaf samples. These regions are characterized by aromatic skeletal vibration of C=O stretch at 1500 cm^{-1} and stretching C-H vibrations around 1233 cm^{-1} of lignin, respectively (Rana et al., 2008; Carballo-Meilán et al., 2014; Shi et al., 2012; Leopold et al., 2011).

The principal wavenumbers found in the analysis of stem bark were 750, 1030, and 1596 cm^{-1}. Absorption bands in these regions are usually due to C-H deformation in cellulose and hemicellulose (Pandey and Pitman, 2003). The peaks from root bark were in the bands around 1030, 1125 and the 1650 cm^{-1}. Peaks in those regions are characteristic of lignin C-H and aromatic skeletal vibration combined with C-H in-plane deforming and stretching (Rana et al., 2010; Shi et al., 2012; Lammers, 2008; Carballo-meilan et al., 2014). These results indicate all cell wall constituents from leaf, stem bark and root bark in the region 1800 to 600 cm^{-1}.

Targeted analysis

FTIR spectroscopy multivariate data analysis was used to discriminate *Z. zanthoxyloïdes* plant parts under vanillic acid characteristic signals found in the infrared study. As shown in Figure 5a, the two first PCs explained 71% of the spectra dataset variance (PC1: 51.56%; PC2: 20.30%) and PC2 explained mainly the discrimination between clusters. Indeed, most of the stem bark and root bark samples clustered closely together regardless of the sampling site on the positive side of the PC2 axis. In contrast, the leaves samples from the various collection sites built up a close group on the negative side of the PC2 axis. The loading plots under PC2 were analyzed to

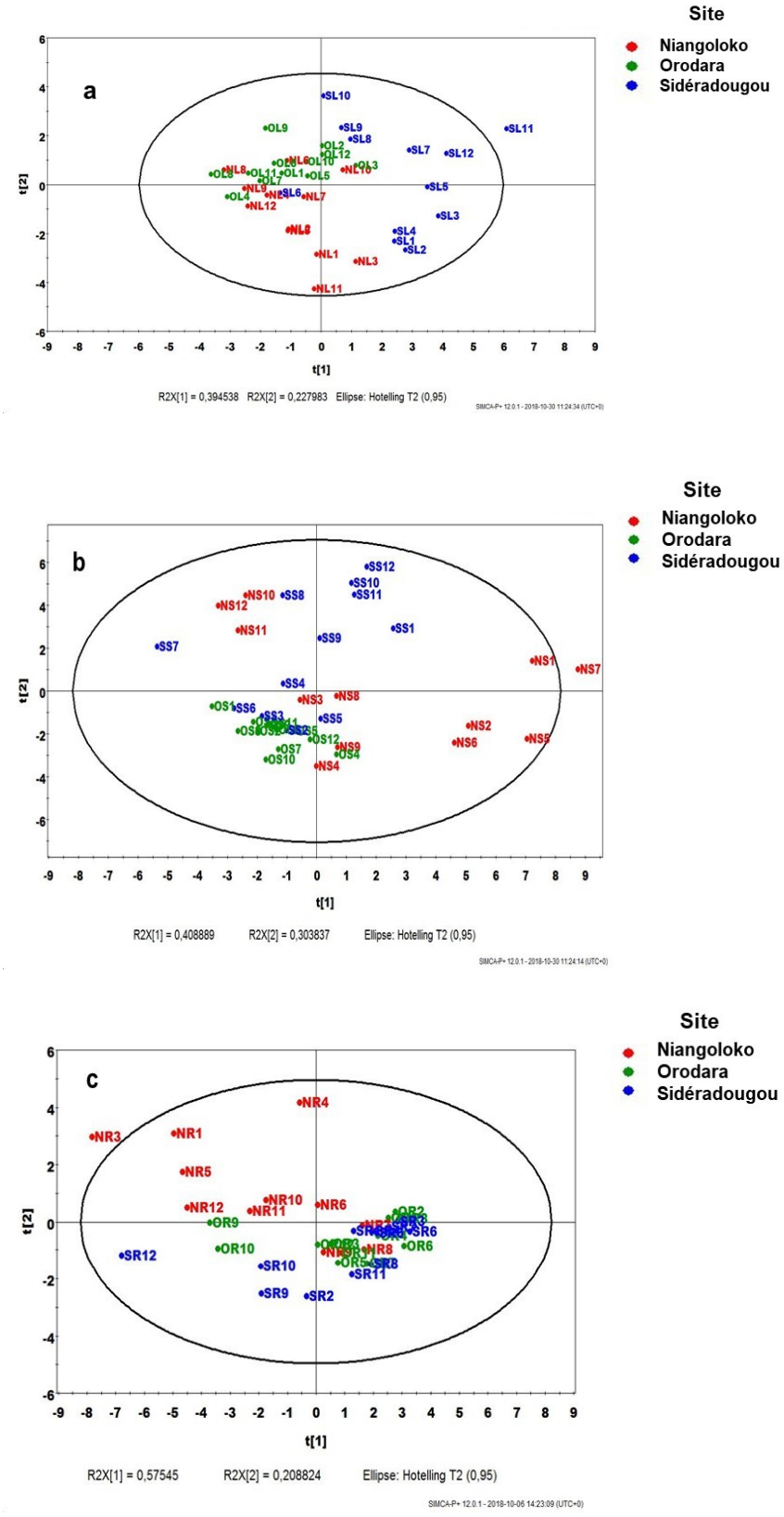

Figure 3. PCA analysis of *Z zanthoxyloïdes* leaf (a), stem bark (b) and root (c) in the fingerprint region (1800-600 cm^{-1}). O for Orodara, S for Sidéradougou, N for Niangoloko. Plant parts: L=Leaf, S= stem bark, R= Root. The colors refer to sites

Table 2. The one-way ANOVA analysis of PCA scores based infrared signals in the range 1800-600 cm^{-1}

Organs	DF	F	*p*-value	Signification
Leaf	35	19.48	<0.001	HS
Stem bark	35	5.26	0.01	S
Root bark	35	1.72	0.19	NS

DF: Degree of freedom, F: Fisher coefficient, HS: highly significant, S: significant, NS: non-significant.

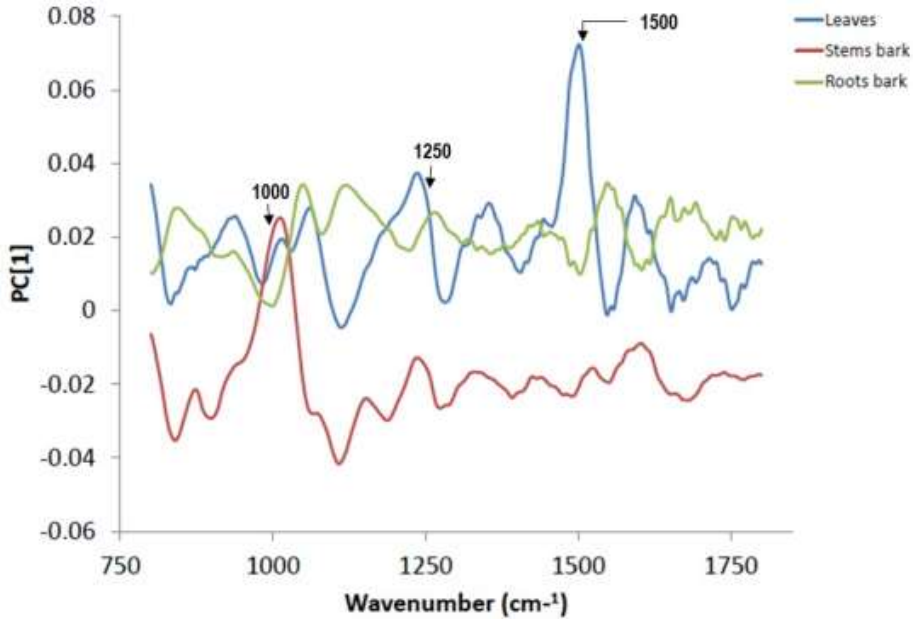

Figure 4. The loadings profiles of the first principal component (PC1) for leaf, stem barks and roots in the region (1800-600 cm^{-1}). The colors refer to plant parts.

highlight the characteristic signals. Examination of the PC2 loadings plot (Figure 5b) showed that the variance along this component is mainly driven by signals around 1030 and 1100 cm^{-1}. The bands in the region 1030 cm^{-1} region are due to aromatic C-H in plane deformation, guaiacyl-type, C-O deformation, and primary alcohols (Rana et al., 2008), while those in the region 1100 cm^{-1} can be assigned to C–O and C–C stretching modes (Nik Norulaini et al., 2011; Leopold et al., 2011).

With the particularity of spectra in the region around 1030 cm^{-1} (Rana et al., 2008), the relative average content of vanillic acid in plant parts (Figure 6) was evaluated according to signal strength in this region. The results showed that the relative amount of vanillic acid in stems and roots bark was relatively similar within an absorbance range of 0.4 to 0.95 with plus two standard deviations (+2SD) above the average, while leaves contained less vanillic acid within an absorbance range of 0.1 to 0.4 with two standard deviations (-2SD) below the average (Figure 6).

The present results can confirm the widespread use of roots bark for phytomedicine purposes containing vanillic acid related to sickle cell disease. Several studies have already shown that the active principle vanillic acid from *Z. zanthoxyloïdes* was mainly found in the roots bark (Ejele et al., 2012; Ameh et al., 2012; Adegbolagun and Olukemi, 2010; Ouattara et al., 2009; Elekwa et al., 2005). Then, referring to the present work, stem bark can be used as an alternative to root bark and avoid the uprooting of the species that will contribute to its sustainable management in its environment.

Conclusion

Using FTIR based metabolomics on *Z. zanthoxyloïdes* samples (leaf, stem bark and root bark) from three different sites in the south-western region of Burkina Faso, differences among plant specimens were highlighted. Leaf spectra were highly different (p =

Table 3. Band assignments in the mid-infrared region of *Z. zanthoxyloïdes* (leaf, stem bark and root bark)[1].

Wavenumber (cm^{-1})	Band origin	Organs
1748	Hydroxyl groups	Root bark
1730	C=O stretch in unconjugated ketones, carbonyls and in ester groups	Leaf
1696	Aromatic skeletal vibration plus C=O stretch, Aldehydes, Ketones, Carboxylic acids, Esters	Root bark
1650	Aldehyde (CHO) (lignin)	Root bark
1596	Aromatic skeletal vibration plus C=O stretch	Stembark
1596	Aromatic skeletal vibration plus C=O stretch	Leaf
1550	NO2 , Nitro compounds	Root bark
1519	Aromatic skeletal vibrations	Stem bark
1500	Aromatic skeletal vibration plus C=O stretch	Leaf
1429	Aromatic skeletal combined with C-H in-plane deforming and stretching	Root bark
1425	Aromatic skeletal vibrations combined with C-H in plane deformation	Stem bark
1350	C-O stretching vibration (cellulose and hemicellulose)	Leaf
1330	S ring plus G ring condensed	Stem bark
1256 - 1376	The C-H vibrations of lignin	Root bark
1233	C-O stretching, The C-H vibrations of lignin,	Stem bark and leaf
1184	Typical for HGS lignins; C=O in ester groups	Stem bark
1125	aromatic skeletal and C-O stretch	Root bark
1100	C-O-C, C-O dominated by ring vibration of carbohydrates	Stem bark
1060	C-O stretching vibration (cellulose and hemicellulose)	Leaf
1030	Aromatic C-H in plane deformation, guaiacyl type and C-O deformation, primary alcohol, C-O-C, C-O dominated by ring vibration of carbohydrates	Stem bark and root bark
930	No information available	Root bark
926	No information available	Leaf
875	No information available	Stem bark
840	Aromatic C-H out-of-plane deformations of the 1, 3,4,5-substituted rings associated with the syringyl nuclei	Root bark
800	No information available	Leaf
750	Cutin	Stem bark

[1]Band assignment based on Carballo Meilán et al. (2014), Rana et al. (2008, 2010), Lammers (2008), and Pandey and Pitman (2003). FTIR : Fourier transform infrared.

$2.59\times10^{-6} < 0.001$) among the three sites, as were stem bark spectra ($p = 0.01 < 0.05$) among the collection sites while root bark spectra were almost identical ($p = 1.72$) in the three provenances.

The discrimination of samples under vanillic acid characteristic signals, metabolite known to be important for the sickle cell disease treatment, on the other hand, was not correlated to geographic sites, but to plant organs. Root bark and stem bark under vanillic acid characteristic signals generated similar peaks around wavenumber 1030 cm^{-1} within an absorbance range of +2SD from average. Thus, stem bark could serve as a substitute for root as a resource for generating active ingredients from *Z. zanthoxyloïdes,* for the treatment of sickle cell disease. This would avoid detrimental uprooting of *Z. zanthoxyloïdes* plant populations and thus decisively improve the sustainable management of this species in its native environment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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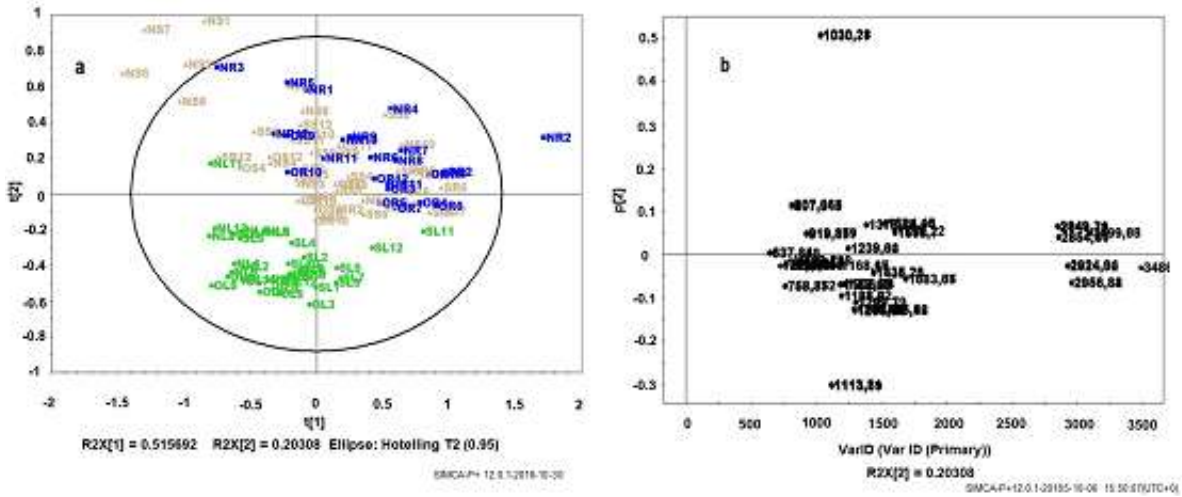

Figure 5. PCA score plot of *Z.zanthoxyloïdes* plant parts (leaf, stem bark and root) under acid vanillic signals (a), Loading plot of spectral variables corresponding to PC2 (b). O for Orodara, S for Sidéradougou, N for Niangoloko. Plant parts: L=Leaf, S= stem bark, R= Root bark. The colors refer to the plant parts.

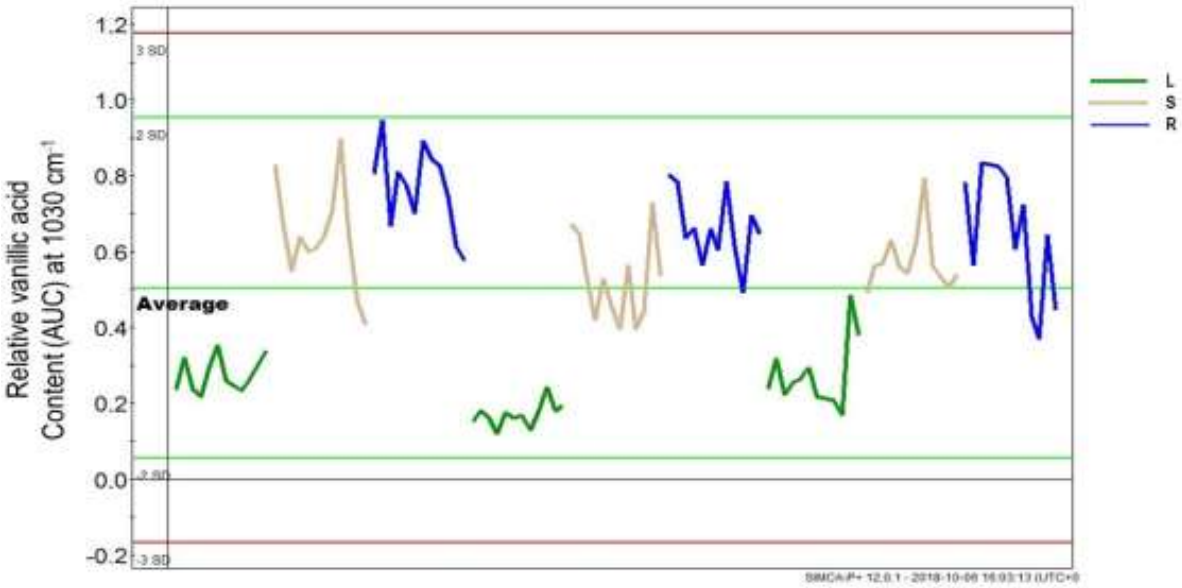

Figure 6. Relative vanillic acid content in plant parts at 1030 cm^{-1}. AUC: Area under curve. The color refers to plant parts. SD: standard deviation. L=Leaf, S= stem bark, R= Root bark.

research work. They also want to extend their heartfelt thanks to the staff and doctoral students of Laboratory of Biochemistry and Bioactive Natural Substance from University Abomey Calavi (Benin) for their help during the recording of the FT-IR spectra of the samples.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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