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Research Article

Solvents, Extraction Method and Plant Parts Influence on Phenolic Compound Extracts and Antioxidant Activity of *Zanthoxylum zanthoxyloides* (Rutaceae)

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ABSTRACT

Background: The study evaluated total polyphenols, total flavonoids, condensed tannins, and antioxidant activity of *Zanthoxylum zanthoxyloides* plant parts' extracts. Specifically, the extraction method and solvent influences on the amount of bioactive compound extracted were assessed. **Materials and Methods:** In detail, the extraction methods were the decoction (Dec) and the maceration (Mac). Also, 3 solvents were used, whose were distilled water (Dw), 70% methanol (70%Met), and 70% ethanol (70%Eth). The leaves (L) and bark (B) were collected in the wild at Yamoussoukro, Côte d'Ivoire. Thereafter, they were dried in the laboratory, ground, and then subjected to either aqueous decoction or hydroalcoholic maceration. Total polyphenols, total flavonoids, and condensed tannins were then measured spectrophotometrically. Additionally, antioxidant activity was assessed using $ABTS^{\cdot+}$ cation. **Results:** The results revealed significant variation in secondary metabolites' contents. For example, leaves and bark decoction yielded similar polyphenol values for 108.29±9.94 and 105.35±9.94 mg GAE/kg (*p*=0.837). When it came to the solvents effect, Dw with the leaves, bark, and 70%Met with the bark formed a similar group with 114.55±6.67 mg GAE/kg. Looking at total flavonoids, leaves decoction led to 2.08±0.11, while the bark maceration with 70%Met gave 2.27±0.10 mg QE/kg (*p*<0.0001). Also, bark decoction and maceration favored condensed tannins extraction for 27.64±5.77 and 22.92±2.58 µg TAE/kg, respectively (*p*=0.486). Furthermore, 70%Eth was more effective with 33.37±5.47 µg TAE/kg. Finally, the antioxidant activity was the best with leaves decoction, and 70%Met for 96.02±5.49 and 218.69±5.21 µmol Trolox.E/kg, respectively. **Conclusion:** *Zanthoxylum zanthoxyloides'* leaves are good sources of bioactive compounds.

Keywords: Antioxidant activity, Condensed tannins, Total flavonoids, Total polyphenols, *Zanthoxylum zanthoxyloides*.

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INTRODUCTION

Zanthoxylum Zanthoxyloides (Rutaceae) is a medicinal plant in West Africa. It is used in the traditional treatment of numerous ailments such as sickle cell disease, toothache, infections, and inflammations (Alade *et al.*, 2023; Ouédraogo *et al.*, 2024). According to Van-Tine *et al.* (2023), the leaves and bark contain various bioactive secondary metabolites of phenolic compounds such as flavonoids, tannins, and coumarins. These compounds confer significant antioxidant activity (Van-Tine *et al.*, 2023).

Phenolic compounds play a key role in neutralizing free radicals, thus contributing to the prevention of chronic diseases linked to oxidative stress, such as cardiovascular diseases, cancer, and neurodegenerative disorders (Yousefi *et al.*, 2022). Generally, plant extracts' antioxidant activity is evaluated using *in vitro* methods such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferric chloride assays. Reducing Antioxidant Power (FRAP), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and the quantification of total phenolic compounds (TPC) are all measured. Several studies have demonstrated that these compounds extracting efficiency highly depend on the solvent used and the plant part analyzed (Yazdani *et al.*, 2021; Alade *et al.*, 2023). Indeed, hydro-alcoholic solvents such as 70% methanol or ethanol mixtures were more effective than water or pure alcohols for extracting a broad spectrum of bioactive compounds (Yazdani *et al.*, 2021; Yousefi *et al.*, 2022). Considering *Z. zanthoxyloides*, the leaves have been found to be richer in phenolic compounds than the bark (Ouédraogo *et al.*, 2024). Since the plant parts bioactive compound contents depend on the soil on which the plant is grown, the present study characterized the phenolic compounds present in *Z. zanthoxyloides*' leaf and bark extracts,

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around Institut National Polytechnique Felix Houphouet Boigny (INP-HB) in Yamoussoukro. As elsewhere, the essay assumed that the plant parts, the solvents and the extraction mode may have significant effect on the bioactive compound extracts. These extracts were obtained by decoction and maceration, using the plant leaves and bark. The assay also evaluated their antioxidant activity in relation to 3 types of solvents, like distilled water, 70% methanol (70%Met), and 70% ethanol (70%Eth). Finally, the study analyzed the interaction between the solvent and the organ to optimize the extractions.

The overall objective was to evaluate *Z. zanthoxyloides* leaves and barks extract phenolic compound contents, and antioxidant activity, obtained by decoction and maceration. Specifically, the objectives were (1) to quantify the phenolic compounds in the leaves and barks extracts; (2) to compare the decoction and the maceration effects on the phenolic compound extracts; and (3) to investigate these extracts' antioxidant activity by using $ABTS^{\cdot+}$ cation.

MATERIALS AND METHODS

The material such as *Z. zanthoxyloides*' plant parts, and methods used are addressed.

Plant Parts

Z. zanthoxyloides' leaves and bark were collected from the bush nearby the Graduate School of Agronomy (ESA) at Institut National Polytechnique Félix Houphouët-Boigny (INP-HB) in Yamoussoukro city, Côte d'Ivoire. Then, they were dried indoors at room temperature for two weeks and ground into a powder using a grinder (Nefret, China). The powder from each part was stored in an airtight container at room temperature.

Chemical Compounds Extraction

The extractions were carried out by decoction and maceration. By decoction, 1 gram of powder from each sample was added to 120 mL of distilled water (Shewale and Rathod, 2018). The mixture was boiled in a water bath for 60 min, including 30 min for boiling and 30 min for cooling. The broth was then filtered through a clean cloth. The resulting filtrates were placed in test tubes and centrifuged at 4000 rpm for 2 min and stored at 4°C until analyses. For the maceration, 1 g of powder from each sample was added to 60 mL solvent such as distilled water, 70% methanol, and 70% ethanol (70/30, v/v, alcohol/distilled water). Each mixture was macerated at room temperature for 40 min. After filtration, the filtrates were centrifuged for 2 min at 4000 rpm at room temperature. Finally, the centrifuged filtrates were filtered again with No. 1 filter paper (Moccamaster, Netherlands), and kept at 4°C until the analysis.

Total Polyphenol

For the determination of total polyphenols, Xiang *et al.* (2024) method was used. So, a volume of 2.5 mL Folin-Ciocalteu reagent diluted 1:10 was added to 30 µL of extract. The mixture was incubated in the dark at room temperature for 2 min. Then, 2 mL of sodium carbonate (Na_2CO_3) solution were added. The entire mixture was incubated at 50°C in a water bath for 15 min. After cooling, the absorbance was measured at 760 nm using a spectrophotometer against distilled water blank. A calibration curve was established using gallic acid at different concentrations (Eq.1, Eq.2). Readings were taken in triplicate and polyphenols' contents were expressed in grams per liter of gallic acid equivalent ($GAE.L^{-1}$). These results were converted in gallic acid equivalent per gram (Yao *et al.*, 2023).

$$F_c\left(mg\,\frac{EAG}{g}\right) = C_r * \frac{1}{9.06*10^3} \qquad \text{Equation 1, for macerations}$$

$$F_c\left(mg\,\frac{EAG}{g}\right) = C_r * \frac{1}{2*9.06*10^3} \qquad \text{Equation 2, for decoctions}$$

Fc was the final content in mg GAE/g, Cr was the content read by spectrophotometer in mg GAE/L.

Total Flavonoid

Total flavonoid content was determined according to Marinova *et al.* (2005). In a 25 mL bottle, 0.75 mL of 5% (weight/v) sodium nitrite ($NaNO_2$) was added to 2.5 mL of extract. Then, 0.75 mL of 10% (weight/v) aluminum chloride ($AlCl_3$) was added to the mixture, and the solution was incubated for 6 min in the dark. Next, 5 mL of 1N sodium hydroxide (NaOH) was added, and the volume was brought up to 25 mL. The preparation was shaken before the total flavonoid content was determined by using a UV-visible spectrophotometer at 510 nm. A calibration curve was generated using quercetin at various concentrations (R^2=0.9976). The total flavonoid content was expressed as grams of quercetin equivalent per liter (g QE/L). The results were converted to grams of quercetin equivalent per gram of sample (g QE/g) (Yao *et al.*, 2023, Eq.3, Eq.4).

$$F_c\left(mg\,\frac{EQ}{g}\right) = R_c * \frac{1}{0.6} \qquad \text{Equation 3, for macerations}$$

$$F_c\left(mg\,\frac{EQ}{g}\right) = R_c * \frac{1}{2*0.6} \qquad \text{Equation 4, for decoctions}$$

Fc was the final content in mg GAE/g, Rc was the content read by spectrophotometer in mg GAE/L.

Condensed Tannins

The condensed tannins' contents were determined according to Julkunen-Titto (1985). A 50 mL aliquot of each extract was added to 1500 µL of 4% vanillin in methanol solution. The mixture was stirred, and 750 µL of concentrated hydrochloric acid (37% (m/m)) was added. The mixture was left to stand at room temperature for 20 min, to react. Thereafter, the absorbance was measured at 550 nm against a blank of 4% vanillin in methanol solution. The tests were performed in triplicate for each sample. A stock solution of tannic acid was used as a reference standard for establishing the calibration curve and for quantifying the condensed tannin' contents. The contents were expressed as milligram equivalent of tannic acid per gram of matter (mg TAE/g). The tests were carried out in triplicate for each sample. The condensed tannin content of the hydroalcoholic extract and fractions was determined (Eq.5, Eq.6, Yao *et al.*, 2023).

$$F_c\left(\mu g\,\frac{E.Cat}{g}\right) = C_r * \frac{1}{5.5} \quad \text{Equation 5, for macerations}$$

$$F_c\left(\mu g\,\frac{E.Cat}{g}\right) = C_r * \frac{1}{2*5.5} \quad \text{Equation 6, for decoctions}$$

Fc was the final content in mg TAE/g, Cr was the content read by spectrophotometer in mg TAE /L.

Antioxidant Activity (AOA)

Antioxidant activity (AOA) was determined according to Teow *et al.* (2007) method. The ABTS radical cation was produced by reacting 8 mM ABTS by mixing 87.7 mg in 20 mL of distilled water, with 3 mM potassium persulfate by dissolving 0.0162 g in 20 mL of distilled water, in 1:1 (v/v) ratio. Then, the mixture was incubated in the dark at room temperature for 16 hr. This ABTS solution was diluted with methanol to obtain a solution with an absorbance of 0.7±0.02 at 734 nm. Following, 3.9 mL of the diluted ABTS solution was added to 100 µL of the extract. After stirring, the mixture was incubated for 6 min in the dark in a water bath at 30±2°C. ABTS radical residual absorbance was then measured at 734 nm using a UV-visible spectrophotometer. The tests were performed in triplicate, and the results were expressed in micromoles of Trolox equivalent per liter (µmol Trolox.E/L). The results were converted to micromoles of Trolox equivalent per gram of extract (µmol Trolox.E/g) (Eq.7 and Eq.8; Yao *et al.*, 2023).

$$F_c\left(\mu mol\,\frac{E.Trolox}{g}\right) = C_r * \frac{1}{2.4} \quad \text{Equation 7, for macerations}$$

$$F_c\left(\mu mol\,\frac{E.Trolox}{g}\right) = C_r * \frac{1}{2*2.4} \quad \text{Equation 8, for decoctions}$$

Fc was the final content in µmol Trolox.E/g, Cr was the content read by spectrophotometer in µmol Trolox.E/L.

Statistical Analysis

The data were subjected to an analysis of variance (ANOVA) using XLSTAT software, version 2021.2.2.1141. Test on the normality of the residuals (Shapiro-Wilk) was applied to see if the residuals can allow ANOVA application. The least square means method was applied and the means were separated according to Duncan's test within 95% confidence interval. The quantitative data was the bioactive compound contents, and the qualitative data were the plant' part like the bark and the leaves, the extraction mode such as maceration and decoction, and the solvents like 70%Meth, distilled water, and 70%Eth.

RESULTS AND DISCUSSION

The bioactive like total phenols, total flavonoids, condensed tannins contents, and the antioxidant activity results are provided.

Total Polyphenols

The interactions between the extraction methods, and plant' parts, and the solvents and plant' parts revealed a significant effect on *Z. zanthoxyloides* polyphenol extracts (Figures 1a, b). At the first stage (Figure 1a), when the interaction concerned the extraction modes, whose were the decoction (Dec) or the maceration (Mac), two main groups appeared. For example, through the Dec, the leaves (L) and the bark (B) total polyphenol extracts were 108.29±9.94 and 105.35±9.94 mg GAE/kg of matter, respectively (p=0.837). Following, the maceration of the bark resulted in 20.60 mg GAE/kg below the average of Dec*L and Dec*B which was 106.82 mg GAE/kg). But, Mac*B result 86.22±4.44 mg GAE/kg was like the first group (p=0.098).

However, the leaf maceration had the lowest total polyphenol result for 72.97±4.44 mg GAE/kg. While, this result was not different from the maceration of the bark result (p=0.051), it was different from the leading group average 106.82 mg GAE/kg ($0.023 \le p \le 0.024$). So, in general, the decoction allowed higher extractions for the bioactive compounds, than the maceration. This increase is due to the heat, which breaks down plant parts' cell walls. Thus, it facilitated polyphenols release within the plant structures (Alara *et al.*, 2023). However, excessively high temperatures can also cause heat-sensitive substances degradation such as total flavonoids, hence the importance of maintaining a balance (Liu *et al.*, 2024).

These ongoing results show that with *Z. zanthoxyloides*, the gain from solubilization outweighs the losses due to degradation, explaining the decoction superiority. Similarly, Ibrahim *et al.* (2025) demonstrated that aqueous decoction frequently provides better total polyphenols yield than aqueous maceration. Furthermore, the leaves contain more polyphenols than the bark, whether prepared by decoction or maceration. This difference is related to the higher contents of soluble flavonoids and phenolic acids in leaf tissues. The more lignified bark contains polyphenols often bound to polysaccharides and less accessible to aqueous solvents (Mensah *et al.*, 2024). Again, these observations were consistent with Adepoju *et al.* (2024) findings.

Similarly to Adepoju *et al.* (2024) results, *Z. zanthoxyloides* leaf extracts possess higher total phenolic contents, compared to bark. Furthermore, the interaction between the extraction method and the plant parts is important. Leaves provided the best yields, particularly when they were subjected to decoction. This highlights that the combined choice of the plant part, and extraction method is crucial for maximizing polyphenol release. This result is analogous to Kaur *et al.* (2025) and Zhang *et al.* (2024) findings. Indeed, Kaur *et al.* (2025) and Zhang *et al.* (2024) emphasized the importance of leaf decoction as the most appropriate method for obtaining a polyphenol-rich aqueous extract. However, Zhang *et al*. (2024) suggests that hydroalcoholic solvents use could further increase bioactive compounds recovery.

The results from the interaction between solvents and the plant parts corroborated Zhang *et al*. (2024) suggestions (Figure 1b). For instance, with hydro-alcohols, only the maceration was performed. Remarkably, the bark maceration with 70% methanol hydro-alcohol (70%Met*B) delivered 111.11±9.43 mg GAE/kg. Indeed, this result laid between the distilled water extractions, whose maybe through decoction or maceration. Surely, *Z. zanthoxyloides*' organs polyphenol contents varied significantly (Figure 1b). However, the bark (E) showed the highest yield with 70%Met for 111.11±9.43 mg GAE/kg. This content was higher than that obtained with 70%Met for the leaf 70.64±9.43 mg GAE/kg (p=0.036). In general, the leaves (F) were little influenced by the solvent choice, 78.73±9.43 mg GAE/kg with 70%Eth*L, and 70.64±9.43 with 70%Met*L (p=0.458). The solvent effect depends on the plant matrix. This result is similar to Sun *et al*. (2025) conclusion, who showed that tissue structure and the phenolic compounds localization modulate extraction efficiency. The superiority of 70% Meth with the bark (E) could be explained by this solvent intermediate polarity. In fact, methanol allows both weakly and moderately solubilization polar compounds. This mechanism has been observed in various matrices by Xiang *et al*. (2024). Conversely, the lack of a significant difference for leaf (F) suggests that its polyphenols are more hydrophilic or less bound to the matrix, making the solvent less decisive. Ultimately, the data highlighted a significant interaction between the solvent and the organ. The solvent 70%Meth is more suitable than 70%Eth for *Z. zanthoxyloides* plant parts polyphenols extraction.

Total Flavonoids

Looking at the extraction mode and plant organ effect on total flavonoids extracts, the decoction maximized the total flavonoid extracts. For example, the decoction of leaf (Dec*L) allowed 2.8±0,11 mg QE/kg (Figure 2a). This content remained similar to the decoction of bark (Dec*B) which result was 2.07±0,11 mg QE/kg (p=0.971). Thereafter, the bark maceration (Mac*B) followed with 1.50±0,05 mg QE/kg, which was very low compared to Dec*B result (p=0.000). Finally, compared to any extract, the maceration of leaf (Mac*L) had the lowest contents for 1.2±0,05 mg QE/kg (p<0.0001). Globally, these results were consistent with Zhang *et al*. (2023) ones. In fact, Zhang *et al*. (2023) stated that the decoction increases the diffusion and desorption of cell wall-bound flavonoids, and can hydrolyze glycosides into more extractable aglycones. So, the decoction increases the overall yield for certain matrices, often with a peak in total flavonoids compared to conventional hydroalcoholic extractions (Zhang *et al*., 2023). On the other hand, maceration at room temperature limits the release of some compounds "trapped" in the parietal architecture, which explains the relatively low extractions.

However, the beneficial effect of the heat is not universal. For example, Gao *et al*. (2022) and Sun *et al*. (2024) pointed out that flavonoids are heat-sensitive, and that prolonged or overly intense treatments accelerate oxidation, unwanted glycosidic bond cleavage, or rearrangements, leading to a decrease in some compounds. This could explain why the bark (Dec*B), although improved by the decoction for 2.07±0,11 mg EQ/kg extraction, does not reach the leaf levels (Dec*L). The bark is likely more sensitive to heat due to its composition and structure, while the leaves benefit from a diffusion and hydrolysis mechanism that limits losses due to thermal degradation beyond the optimum (Gao *et al*., 2022; Sun *et al*., 2024).

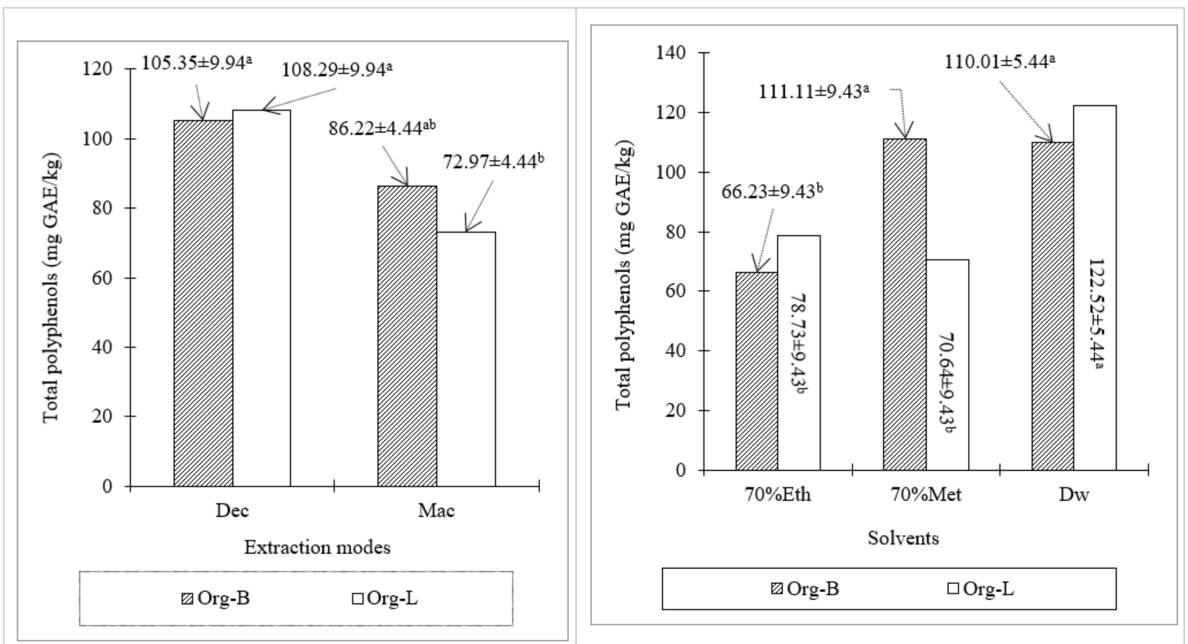
According Okagu *et al*. (2021) and Yang *et al*. (2024), *Zanthoxylum genus* total flavonoid extracts vary between leaves, bark, and fruit. Moreover, the parameters such as solvents, temperature, and time strongly influence the extraction yields (Figure 2b). For instance, Li *et al*. (2024) work on the ultrasound-assisted total flavonoids extraction from *Z. bungeanum* residues and their antioxidant properties confirms this assertion. In practice, decoction appears to be the preferred method for maximizing total flavonoid extraction, because it is the cheapest. Comparatively, the maceration remains useful for heat-sensitive targets, cold standardization, or when regulatory constraints or formulation requirements necessitate gentle processes. According to Chemat *et al*. (2025) intensified "green" processes such as ultrasound make it possible to increase yields at lower temperatures and could offer a compromise between efficiency and selectivity for *Zanthoxylum genus*.

So, through maceration, total flavonoid extraction was influenced by the solvents, the plant part, and the extraction methods (Figure 2b). Total flavonoid extracts varied and presented 3 different groups. The extraction through maceration with hydro-alcohols allowed the best extractions. Surely, 70%Met*B permitted 2.27±0,10 mg QE/kg, and that result was the highest (p<0.0001). Thereafter, the nodes 70%Eth*B, 70%Met*L and 70%Eth*L results were 1.93±0.10, 1.83±0.10, and 1.61±0.10 mg QE/kg, respectively. These results formed a homogenous group with a least square mean of 1.79±0.10 mg QE/kg (0.073≤p≤0.527). At the third position, Dw*L and Dw*B extracts were 1.20±0.06 and 1.16±0.06 mg QE/kg, respectively (p=0.627). These ongoing results were similar to Ouédraogo *et al*. (2024) outputs. These authors demonstrated that moderately polar organic solvents, such as methanol or ethanol diluted in water, improve the phenolic compounds extraction, including total flavonoids (Ouédraogo *et al*., 2024). Methanol's ability to efficiently extract flavonoids is linked to its higher polarity, with 5.1 approximately polarity index compared to ethanol's 4.3. Thus, according to Luo *et al*. (2025) 70%Met was able to solubilize both glycosylated flavonoids and aglycones. In contrast, Ozigis *et al*. (2023) said that distilled water, although very polar, extracts less well the aglycone flavonoids which are less hydrophilic.

To summarize, *Z. zanthoxyloides* bark consistently exhibits higher flavonoid content than the leaves, with the hydro-alcohols. These ongoing findings support Tine *et al*. (2017) conclusion. Indeed, in their analysis of flavonoid compounds in *Z. zanthoxyloides*' extracts, Tine *et al*. (2017) explained that the bark is often an accumulation site of secondary metabolites such as flavonoids, in response to external stresses like ultraviolet radiation and pathogens. The bark acts as a protective barrier, which explains its richness in bioactive compounds. Conversely, with both hydro-alcohols, the leaves total flavonoid extracts were lower than that of the bark. In details, 70%Met*B and 70%Met*L results were 2.27±0.10 and 1.83±0.10 mg QE/kg, respectively (p<0.0001). Again, with 70%Met*B, and 70%Met*L, the results were 1.93 and 1.61 mg QE/kg, respectively. However, flavonoids composition also varies depending on the flavonoid type, as some glycosides are more abundant in the leaves (Ozigis *et al*., 2023). Flavonoid-rich extracts, particularly those from the bark obtained with 70%Met, could be responsible for the biological activities already reported for *Z. zanthoxyloides*, including its antidiabetic, and anthelmintic effects. This assertion supports Azando *et al*. (2022) and Motto *et al*. (2019) position. These authors stated that flavonoids are known for their ability to scavenge free radicals and modulate enzymes involved in inflammation and glucose metabolism (Azando *et al*., 2022; Motto *et al*., 2019).

Condensed Tannins

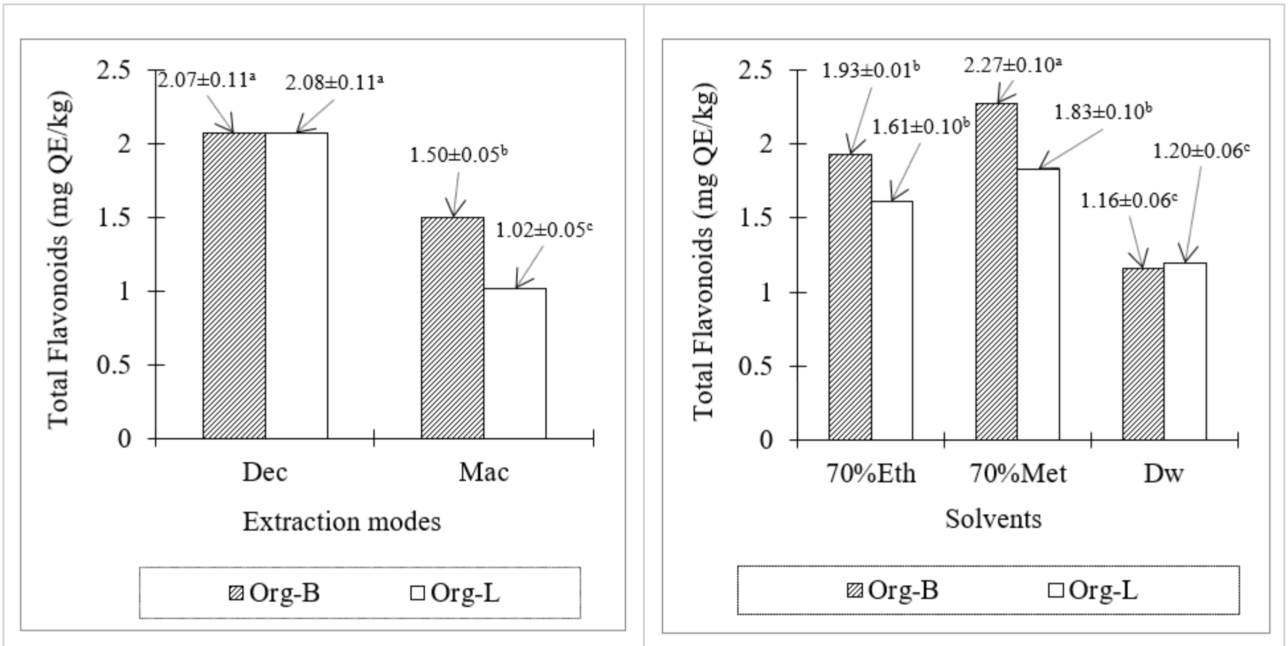
Looking at the extraction mode, condensed tannins extract significantly varied. Remarkably, condensed tannins results followed total polyphenols extract (Table 1a). Namely, the nodes Dec*B; Mac*B, and Mac*L results were 27.64±5.77; 22.92±2.58, and 17.99±2.58 μg TAE/kg, respectively. Altogether, these 3 nodes formed a homogeneous group (0.091≤p≤0.305). So, this first group had an average of 22.85±3.64 μg TAE/kg. Next, the leaves decoction (Dec*F) delivered the lowest extract for 12.13±5.77 μg TAE/kg, compared to bark extracts (p=0.005). This trend suggests a significant interaction between the extraction method and the organ on the condensed tannins extracted amount. This result is similar to Falleh *et al*. (2021) and Cissé *et al*. (2024) findings. They suggested that maceration appears to better preserve heat-sensitive phenolic compounds, such as condensed tannins, allowing for better extraction in leaves that are richer in these compounds (Falleh *et al*., 2021; Cissé *et al*., 2024). Conversely, decoction, which involves exposure to high temperature, can partially degrade tannins, which explains the decrease observed in leaves' decoction extracts. Furthermore, Koutouan *et al.* (2019) found that the plant organ plays a crucial role. For example, leaves, with their more accessible surface and cellular structure, efficiently release tannins during maceration and even decoction (Koutouan *et al*., 2019). In contrast, the bark, being more lignified and robust, releases fewer condensed tannins, particularly



a – Extraction modes*Organs effect on Total polyphenols extracts

b – Solvents*Organs effect on Total polyphenols extracts

Figure 1: *Z. zanthoxyloides*' part total polyphenol extracts. In the same section (a or b), the results labeled with different superscript letters (a or b) differ significantly (p<0.05); Dec: decoction; Mac: maceration; L: leaf; B: bark; Dw: distilled water; 70%Meth: 70% methanol; 70%Eth: 70% ethanol, LSM: least square mean; SE: standard error.



a - Total flavonoid content as a function of the interaction between the extraction method and the organs

b - Total flavonoid content as a function of solvent-organ interaction

Figure 2: Total flavonoid extracts of *Z. zanthoxyloides* plant' parts.

during maceration where the temperature does not facilitate the extraction of these cell-bound compounds.

Additionally, extraction by maceration with 70%Eth applied to the bark (70%Eth*B) yielded 33.37±5.47 µg TAE/kg, the highest content (Table 1b). The node 70%Eth*B shared this leading group with nodes Dw*L, and Dw*B, whose results were 31.45±3.16 and 29.52±3.16 µg TAE/kg, respectively ($0.765 \leq p \leq 0.817$). Thus, this leading group average result was 31.45±3.93 µg TAE/kg. Notably, 70%Meth poorly performed compare to 70%Eth. Next, 70%Met*B, 70%Eth*L, and 70%Met*L outputs were 12.95±5.47, 10.51±5.47, and 8.58±5.47 µg TAE/kg ($0.099 \leq p \leq 0.574$). This second group was significantly lower than the first group ($0.001 \leq p \leq 0.038$). These results reflected a significant interaction between the solvent and the plant organ. The hydro-alcohols 70%Eth and 70%Met are known for their effectiveness in extracting phenolic compounds. The present results were similar to Ncube *et al.* (2021) ones. In particular, tannins, due to their polar and nonpolar properties, allow better solubilization of condensed tannins with distilled water. Furthermore, Baskal *et al.* (2022) stated that the use of hydro-alcoholic solvents like 70%Eth is recommended to maximize tannin extraction in lignified tissues like the bark (B).

Antioxidant Activity

The highest antioxidant activity was obtained with the leaves' decoction (Dec*L) for 96.02±5.49 µmol Trolox.E/kg (Table 2a, $p<0.0001$). Following this highest extraction, the leaves' maceration (Mac*L) led to 85.22±2.45 µmol Trolox.E/kg. In contrast, the antioxidant activity decreased sharply in the bark decoction (Dec*B) and bark maceration (Mac*B) for 23.27±5.49 and 21.85±2.45 µmol Trolox.E/kg, respectively. Regarding the extraction method effect, decoction often leads to better phenolic compounds extraction, particularly from leaves, as reported by (Faye *et al.*, 2022). They also stated that low temperatures can limit extraction in certain matrices, such as the bark, where the structure is denser (Faye *et al.*, 2022). This explains the little difference between maceration and decoction for the bark. Furthermore, leaves (L) generally released more total polyphenolic compounds than the bark (B) for both methods (Figure 1a). This result is similar to that of Zhang *et al.* (2021). Indeed, Zhang *et al.* (2021) stated that the leaves' cellular structure is more accessible and richer in easily extractable polyphenols, while bark is more resistant and requires more intense decoction conditions for maximum extraction. In the whole, more bioactive compound extraction leads to higher antioxidant activity (Figure 1, Table 2).

Comparatively, the solvents play a crucial role in the bioactive compounds extraction and directly influence the antioxidant extracts' activity. For example, the leaves extraction through

Table 1: Extraction mode, plant organ and solvent effects on condensed tannin extractions.

α. Extraction*Organ	µg TAE/kg±SE	β. Solvent*Organ	µg TAE/kg±SE
		70%Eth*B	33.37±5.47[a]
Dec*B	27.64±5.77[a]	Dw*L	31.45±3.16[a]
Mac*B	22.92±2.58[a]	Dw*B	29.52±3.16[a]
Mac*L	17.99±2.58[ab]	70%Met*B	12.95±5.47[b]
Dec*L	12.13±5.77[b]	70%Eth*L	10.51±5.47[b]
		70%Met*L	8.58±5.47[b]
p-value	<0.0001		<0.0001

In the same section (α or β), the results labeled with different superscript letters (a or b) differ significantly ($p<0.05$); Dec: decoction; Mac: maceration; L: leaf; B: bark; Dw: distilled water; 70%Meth: 70% methanol; 70%Eth: 70% ethanol, LSM: least square mean; SE: standard error.

Table 2: Antioxidant activity of *Z. zanthoxyloides* (µmol Trolox.E/kg).

α. Extraction*Organ	Average (µ±SE)		β. Solvent*Organ	Average (µ±SE)
Dec*L	96.02±5.49[a]		70%Met*L	218.69±5.21[a]
Mac*L	85.22±2.45[b]		70%Eth*L	30.52±5.21[b]
Mac*B	21.85±2.45[c]		Dw*L	25.83±3.01[b]
Dec*B	23.27±5.49[c]		70%Eth*B	17.22±3.01[c]
			Dw*B	17.28±5.21[c]
			70%Met*B	10.83±5.21[c]
p-value	<0,0001			<0,0001

In the same section (α or β), the results labeled with different superscript letters (a, b, c) differ significantly ($p<0.05$); Dec: decoction; Mac: maceration; L: leaf; B: bark; Dw: distilled water; 70%Meth: 70% methanol; 70%Eth: 70% ethanol; µ: least square mean; SE: standard error.

the maceration with hydro-alcohols gave the best results. Not only, the extraction with 70%Met yielded the highest extract for 218.69±5.21 µmol Trolox.E/kg ($p<0.0001$), but also 70%Eth followed with 30.52±5.21 µmol Trolox.E/kg ($p<0.0001$). Distilled water, considering the decoction and the maceration, showed a similar value for the leaves with 70%Eth, and delivered 25.83±3.01 µmol Trolox.E/kg ($p=0.189$). By comparison, extraction with 70%Eth, distilled water (Dw) and 70%Eth of the bark showed a much lower antioxidant activity for 17.22±3.01, 17.28±5.21 and 10.83±5.21 µmol Trolox.E/kg, with an average of 15.11±4.47 µmol Trolox.E/kg.

Facing the hydro-alcohol performance, like 70%Met, this result was consistent with those of Ogundare *et al.* (2025) and Li *et al.* (2024). They indicated that hydroalcoholic solvents extract compounds better than pure solvents such as distilled water (Ogundare *et al.*, 2025; Li *et al.*, 2024). Furthermore, He *et al.* (2024) and Suman *et al.* (2023) showed that leaves, which are likely richer in phenolic compounds such as alkaloids, flavonoids, and tannins, exhibit superior antioxidant activity compared to the bark. Also, this result is similar to Ogundare *et al.* (2025) conclusion. The leaves exhibited greater antioxidant activity than the bark. Similarly, Li *et al.* (2024) explained that the variations in antioxidant activity are likely related to the higher concentration of flavonoids, alkaloids, and tannins in the leaves. The bark extracts that produce lower antioxidant activities suggest a different chemical composition, and are potentially less rich in phenolic compounds, according to Olusola *et al.* (2023). In fact, during the same extraction duration under decoction, the bark may deliver much more polyphenols. But, during short duration like 30 min, the leaves deliver more compounds. Nonetheless, during the same short duration of extraction, hydro-alcohols are the most efficient (Shewale and Rathod, 2018).

CONCLUSION

In the aim to assess *Zanthoxylum Zanthoxyloides* plant parts potential uses for animal gastrointestinal helminthic threat, its leaves and bark were extracted. The extractions were performed by decoction and maceration, with distilled water and hydro-alcohols like 70%Met, and 70%Eth. While distilled water was used for decoction and maceration, the hydro-alcohols were only used for maceration. On the overall, the leaves were richer in total polyphenols and total flavonoids than the bark, confirming their role as a preferred source of bioactive compounds. Decoction yielded the polyphenols and total flavonoids highest contents, particularly in the leaves, while maceration preserved the condensed tannins, especially in the leaves. Regarding the solvents influence, hydro-alcohols, including 70%Met and 70%Eth, gave better yields than distilled water, particularly for the bark. Regarding the antioxidant activity, the leaves confirmed their superiority over the barks, with maximum effectiveness observed for maceration and decoction.

These results highlight the combined choice of plant organ, solvent, and extraction method importance to optimize the bioactive compounds' extraction. They also confirm traditional knowledge by promoting decoction as an effective method for obtaining extracts rich in polyphenols. Nevertheless, certain limitations, such as the thermal degradation of some sensitive compounds, suggest the need for further research. Looking ahead, it would be relevant to explore the "green" extraction processes such as ultrasound, microwaves, and supercritical fluids to improve yields while preserving the metabolites' integrity. More detailed characterization by chromatography or mass spectrometry would allow the compound identification, in *Z. zanthoxyloides* plant parts.

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ABBREVIATIONS

Eth: 70% ethanol; **70%Met:** 70% methanol; **ABTS:** 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid; **ANOVA:** analysis of variance; **AOA:** Antioxidant activity; **B:** bark; **Dec:** decoction; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **Dw:** distilled water; ESA: Ecole Supérieure d'Agronomie; **FRAP:** Ferric Reducing Antioxidant Power; **GAE:** gallic acid equivalent; INP-**HB:** Institut National Polytechnique Felix Houphouet Boigny; **L:** leaves; **LSM:** least square mean; **Mac:** maceration; **QE:** quercetin equivalent; **SE:** standard error; **TAE:** tannic acid equivalent; **TPC:** total phenolic compounds; **Trolox.E:** Trolox equivalent

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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