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PHYTOCHEMICAL INVESTIGATIONS AND EVALUATION OF THE ANTIOXIDANT EFFICACY OF HYDRO-ETHANOLIC EXTRACTS OF *ZANTHOXYLUM ZANTHOXYLOIDES* LAM. FROM KORHOGO, CÔTE D'IVOIRE: COMPARATIVE ANALYSIS OF LEAVES AND STEM BARK

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ABSTRACT

Zanthoxylum zanthoxyloides Lam., also known as *Fagara zanthoxyloides* Lam., a plant used in traditional medicine in Korhogo, a city in Côte d'Ivoire, was the subject of an in-depth study on the hydro-ethanolic extracts of its leaves and stem bark. This study included a phytochemical screening, UPLC-MS analysis, and antioxidant evaluation. Phytochemical screening revealed the presence of tannins, flavonoids, coumarins, sterols, terpenes, volatile oils, and reducing sugars in both plant parts, with anthocyanins specifically detected in the leaves. UPLC-MS analysis identified a flavonol ($C_{15}H_{10}O_3$) in the stem bark. The concentrations of total phenolic compounds, total flavonoids, and condensed tannins were significantly higher in the leaves (530.575 ± 2.341 mg GAE/g DW, 13.18 ± 0.96 mg QE/g DW, and 46.75 ± 3.38 mg CE/g DW) compared to those observed in the stem bark (294.103 ± 8.936 mg GAE/g DW, 7.48 ± 0.30 mg QE/g DW, and 5.31 ± 0.48 mg CE/g DW). The antioxidant

activity of the leaves (IC_{50} = 0.076 mg/mL) significantly surpassed that of the stem bark (IC50 = 0.165 mg/mL).

KEYWORDS: UPLC-MS analysis identified a flavonol ($C_{15}H_{10}O_3$) in the stem bark.

INTRODUCTION

The Ivorian flora represents a valuable resource for traditional medicine.[1] According to data from the World Health Organization (WHO), approximately 80% of the population in developing countries rely on traditional medicine, both to compensate for the difficulty in accessing modern healthcare and as an alternative to it.[2] Herbal medicine presents a promising approach in addressing the drawbacks of synthetic drugs, such as their potential toxicity, the increasing resistance of pathogens, the high cost of modern treatments, and the shortage of healthcare centers, particularly in rural areas.[3] In this context, this study aims to promote Ivorian medicinal plants, with a particular focus on *Zanthoxylum zanthoxyloides* Lam., a species frequently used in traditional medicine in northern Côte d'Ivoire. The efficacy of medicinal plants is often attributed to their secondary metabolites, such as phenolic compounds, alkaloids, and essential oils. Phenolic compounds, in particular, are known for their ability to inhibit certain enzymes and for their antioxidant properties, which justifies their therapeutic use.[4] The objective of this research is to conduct a phytochemical and biological screening to confirm the medicinal properties attributed to this plant in the literature.

MATERIALS AND METHODS

Plant Material

The plant material studied, consisting of the leaves and stem bark of *Zanthoxylum zanthoxyloides*, was collected in February 2023 in Korhogo (Côte d'Ivoire, at the botanical garden of Peleforo Gon Coulibaly University). After identification by botanists from the university, the samples were dried for 7 days at room temperature, then ground and sieved to obtain fine powders used for the preparation of the extracts to be tested.

METHODS

Extractions

After filtration, the filtrates for each plant part were combined and stored in a refrigerator at 4°C for 24 hours to allow the precipitation of lipophilic compounds. A mass of 5 g of powder from each organ (leaves and stem bark) of *Zanthoxylum zanthoxyloides* was macerated in 70

mL of an ethanol/water mixture (49/21) for 24 hours, with three repetitions for each plant part. After decantation and filtration, each hydro-ethanolic extract was divided into two portions. The first portion was used for test-tube assays, including phytochemical screening and antioxidant activity evaluation. The second portion was dried at 50°C for 2 days to obtain hydro-ethanolic extracts. These dried extracts were then used to quantify certain secondary metabolites, determine chemical composition via UPLC-MS, and evaluate antioxidant activity by spectrophotometry.

Phytochemical Screening in Test Tubes

Phytochemical screening is a qualitative technique that allows the identification of different chemical groups contained in a plant organ. The analytical techniques used rely on classical methods commonly applied for the detection of chemical groups in plant extracts.[5,6]

Tannin Detection Test

In test tubes containing 5 mL of each extract, a few drops of 1% aqueous ferric chloride solution are added. The presence of gallic or catechin tannins is indicated by the development of a greenish or bluish-black coloration.

Flavonoid Detection Test

In test tubes containing 5 mL of each extract, a few drops of concentrated hydrochloric acid and 3 magnesium chips are added. The presence of flavonoids in the extracts is indicated by a color change to red or yellow.

Coumarin Detection Test

To 5 mL of each extract in test tubes, 10 drops of 10% KOH are added. The resulting solution is neutralized with 10% HCl solution. The formation of a cloudiness or precipitate in the tubes confirms the presence of coumarins in the extracts.

Free Quinones Detection Test

A few drops of 1% sodium hydroxide are added to test tubes containing 5 mL of the various extracts. The appearance of a yellow-green coloration indicates the presence of free quinones.

Anthraquinones Detection Test

A few drops of 10% ammonium hydroxide are added to test tubes containing 5 mL of each extract. The presence of anthraquinones is confirmed by a color change of the solution to yellow.

Anthocyanin Detection Test

A few drops of 10% sulfuric acid and a few drops of 10% ammonium hydroxide are added to 5 mL of each extract. The presence of anthocyanins is indicated by a black coloration.

Sterols and Triterpenes Detection Test

A reagent consisting of an equal volume mixture of acetic anhydride and sulfuric acid is prepared. A few drops of this reagent are added to 5 mL of each extract. After 15 minutes of incubation, the appearance of a green or purple (violet) color indicates a positive test.

Saponin Detection Test

After adding 5 mL of distilled water to 5 mL of each extract, the test tubes are sealed and vigorously shaken for 15 seconds. The formation of a persistent foam higher than 1 cm indicates the presence of saponins.

Alkaloid Detection Test

To 5 mL of each extract in test tubes, a few drops of HCl and Dragendorff's reagent are added. The presence of alkaloids is observed by the formation of an orange precipitate.

Volatile Oil Detection Test

A few drops of 10% sodium hydroxide and a few drops of 10% hydrochloric acid are added to 5 mL of each extract. The presence of volatile oils in the extracts is indicated by the appearance of a blackish color.

Reducing Compound Detection Test

Fehling's reagent is prepared from liquor A (a mixture of copper sulfate and water) and liquor B (composed of tartaric acid ($C_4H_6O_6$), sodium hydroxide (NaOH), and water). A few drops of Fehling's reagent are added to 5 mL of each extract. The appearance of a brick-red precipitate indicates a positive test.

Determination of Total Phenolic Compounds, Total Flavonoids, and Total Condensed Tannins

Determination of Total Phenolic Compounds

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method.[7] To 1 mL of each extract, 1.5 mL of Na_2CO_3 (17%, w/v) and 0.5 mL of Folin-Ciocalteu reagent (0.5 N) were added. The mixture was incubated at 37°C for 30 minutes, and the absorbance was measured at 760 nm against a blank without extract as the reference. The

quantification of total phenolic compounds was based on a linear calibration curve (y = ax + b) constructed with a standard solution of gallic acid at different concentrations (0 to 1000 µg/mL) under the same conditions as the sample. Results are expressed in milligrams of gallic acid equivalent per gram of dry matter (mg GAE/g DM). The total phenolic content (TPC) is calculated using the following formula.

T_{PT} = Ce×d /Ci (mg GAE /g dry matter

Ce: Concentration of the extract in gallic acid equivalent (mg GAE/mL)

Ci: Concentration of the extract (g/mL)

d: Dilution factor

T_{PT}: Total phenolic content

Determination of Total Flavonoids

This determination was carried out according to the method used by Arvouet-Grand and collaborators.[8] 500 µL of 2% aluminum chloride ($AlCl_3$) in methanol were added to an equal volume of extract. After 10 minutes of incubation, the absorbance was measured at 415 nm using a spectrophotometer. Quercetin (0-100 µg/L) was used as the standard to establish the calibration curve. Three (3) replicates were performed for each extract, and the result provided is an average of the three readings. Results, expressed in milligrams of Quercetin Equivalent (QE) per gram of dry matter (mg QE/g DM), are determined using the following formula.

T_{FC} = Ce×d /Ci (mg QE /g dry matter)

Ce: Concentration of the extract in quercetin equivalent (mg QE/mL)

Ci: Concentration of the analyzed extract (g/mL)

d: Dilution factor

T_{FC}: Total flavonoid content

Determination of Total Condensed Tannins

The determination of condensed tannins in the various extracts was carried out according to the method described by Heimler et al.[9] For each sample or standard, 400 µL was mixed with 3 mL of a 4% methanolic solution of vanillin and 1.5 mL of concentrated hydrochloric acid. The mixture was incubated for 15 minutes, and absorbance was measured at 500 nm. The concentrations of condensed tannins were determined using calibration curves established with catechin (0-300 µg/mL) and are expressed in milligrams of Catechin Equivalent per gram of dry matter (mg CE/g DM).

T_{TC} = Ce×d /Ci (mg CE /g dry matter

Ce: Concentration of the extract in catechin equivalent (mg CE/mL)

Ci: Concentration of the analyzed extract (g/mL)

d: Dilution factor

T_{TC}: Total condensed tannin content

Characterization of Hydro-Ethanolic Extracts of *Zanthoxylum zanthoxyloides* Leaves and Stem Bark by UPLC-MS

The UPLC-MS analysis was performed using a Waters ACQUITY UPLC-MS system. This system includes a quadrupole detector, a mass spectrometer equipped with an electrospray ionization interface (ESI), and a diode array detector. For the analysis, 1 mg of each crude hydro-ethanolic extract was dissolved in 1 to 1.5 mL of analytical-grade methanol. The resulting suspension was filtered, and an adequate amount of the filtrate was placed into a vial specifically designed for the analysis to determine the chromatographic profile of the extract and the mass spectra of the associated compounds. The binary solvent system used consisted of solvents A and B, where A was H_2O/acetonitrile 98/2 + 0.1% formic acid, and B was acetonitrile 100% + 0.1% formic acid. To identify the compounds, the empirical formulas of several secondary metabolites were provided. The presence of secondary metabolites is confirmed by the appearance of one or more green peaks, whereas their absence is indicated by the appearance of red peaks.

Antioxidant Activity

Antioxidant Activity in Test Tubes

The method proposed by Popovici *et al.*[10] was used for the test tube evaluation of antioxidant activity. In a volume of 5 mL of hydro-ethanolic extract solution from each plant organ, 1.5 mL of purple DPPH solution was added. A positive reaction was indicated by the appearance of a yellow color in the medium after 15 minutes of incubation. This color change results from one or more redox reactions between the compound(s) in the tested extract and the free radicals in the DPPH solution.

Antioxidant Activity by Spectrophotometry

The antioxidant potential of the extracts was evaluated using the method of Blois.[11] DPPH was dissolved in absolute ethanol to obtain a solution with a concentration of 0.03 mg/mL. Different concentrations of the extract (2 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, and 0.0625 mg/mL) were prepared in absolute ethanol. In sterile, dry test tubes, 2.5

mL of plant extract and 1 mL of ethanolic DPPH solution were added. After shaking, the tubes were kept in the dark for 30 minutes. The absorbance of the mixture was then measured at 517 nm against a blank composed of 2.5 mL of absolute ethanol and 1 mL of DPPH solution. The reference positive control was ascorbic acid (vitamin C). The DPPH inhibition percentages were calculated using the following formula:

$I(\%) = (A_b - A_e) / A_b \times 100$

Where:

I: Inhibition percentage

A_b: Absorbance of the blank

A_e: Absorbance of the sample

The concentrations required to scavenge 50% of the DPPH (IC_{50}) were determined from the graphs representing the DPPH inhibition percentage as a function of the extract or vitamin C concentrations.

Statistical Analysis of Data

The analysis of the measurements obtained during the different procedures was performed using EXCEL 2021 (version 16.0). This software was used to determine the quantities of total phenolic compounds, total condensed tannins, and total flavonoids in the various extracts. Additionally, it was used to generate the graphs that automatically allowed for the determination of the IC_{50} parameter for each extract.

RESULTS

Yields of the Extractions

The extractions performed yielded hydro-ethanolic extracts from the various studied parts of *Zanthoxylum zanthoxyloides*. The extraction yields were calculated using the following formula.

$$100 \times \frac{\text{extracted mass}}{\text{Initial mass}}$$

The obtained values are shown in Table 1.

Table 1: Yields of hydro-ethanolic extracts from the leaves and stem barks of *Zanthoxylum zanthoxyloides.*

Extraits	**ZZF**			**ZZT**		
Mi (g)	5	5	5	5	5	5
Mex (g)	1,20	1,49	1,31	1,17	1,19	1,27
R (%)	24,00	29,80	26,20	23,40	23,80	25,40
Rm (%)	**26,67±2,93**			**24,2±01,06**		

Mi: initial mass, **Mex**: extracted mass, **R**: yield, **Rm**: average yield, **ZZF**: leaves of *Zanthoxylum zanthoxyloides*, **ZZT**: stems of *Zanthoxylum zanthoxyloides.*

Phytochemical Screening in Test Tubes

The phytochemical screening conducted on the hydro-ethanolic extracts from the leaves and stem barks of *Zanthoxylum zanthoxyloides* provided information on the presence or absence of several chemical compounds. The results of this phytochemical analysis are presented in Table 2.

Table 2: Phytochemical Screening in Test Tubes.

	Different hydro-ethanolic extracts from the organs	
Chemical Compounds Searched	**ZZF**	**ZZT**
Tannins	+	+
Flavonoids	+	+
Coumarins	+	+
Free Quinones	-	-
Anthraquinones	-	-
Alkaloids	+	+
Sterols and Terpenes	+	+
Saponins	-	-
Anthocyanins	+	-
Volatile Oils	+	+
Reducing Sugars	+	+

ZZF: Leaves of *Zanthoxylum zanthoxyloides*; ZZT: Stem barks of *Zanthoxylum zanthoxyloides*. -: absence, + : presence

UPLC-MS Analysis of Hydro-Ethanolic Crude Extracts from the Leaves and Stem Barks of *Zanthoxylum zanthoxyloides*

The characterization of the five major compounds from the hydro-ethanolic extracts of the leaves and stem barks of *Z. zanthoxyloides* was carried out using Ultra-Performance Liquid Chromatography coupled with Mass Spectrometry (UPLC-MS). This analysis is illustrated in Figure 1, which shows the chromatographic profile of the hydro-ethanolic extract from the stem barks of *Z. zanthoxyloides*. Table 3 provides the retention time (Tr) and the percentage

presence (P) of each compound in the various studied extracts. The percentage presence of the compounds ranges from 8.06% to 48.22%, and their retention times range from 0.78 to 3.44 minutes. The analysis of the compounds was performed by comparing the mass spectra of standard molecules with those of each compound. For instance, a flavonol from the flavonoid family (peak 1) with a molecular mass of 238.06 and a formula of $C_{15}H_{10}O_3$, represented in green in Figure 1, was identified in the hydro-ethanolic extract of the stem barks of *Z. zanthoxyloides*.

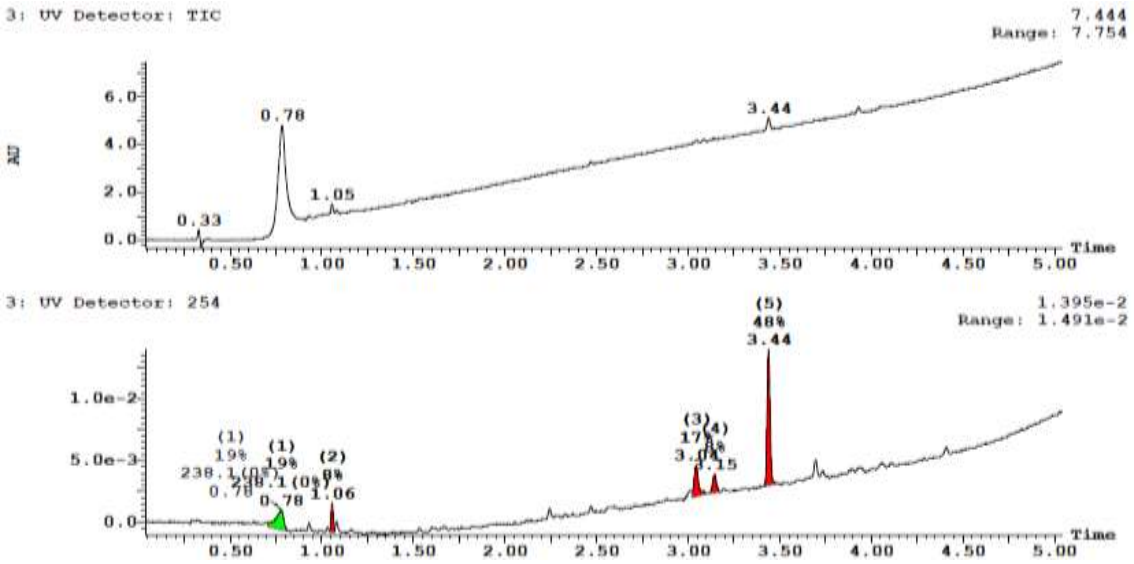

Figure 1: Chromatogram of the hydro-ethanolic extract from the stem barks of *Z. zanthoxyloides*.

Table 3: Identification of the five major compounds from the leaves and stem barks of *Z. zanthoxyloides*.

	ZZF					ZZT				
	Peak 1	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**	**Peak 1**	**Peak 2**	**Peak 3**	**Peak 4**	**Peak 5**
Tr (mn)	0.78	1.54	3.01	3.04	3.14	0.78	1.06	3.04	3.15	3.44
P (%)	31.95	16.70	8.43	29.89	15.02	18.98	8.06	16.52	8.22	48.22
Compound	NI	NI	NI	NI	NI	**Flavonol**	NI	NI	NI	NI
Formula	NI	NI	NI	NI	NI	**$C_{15}H_{10}O_3$**	NI	NI	NI	NI
M (g/mol)	NI	NI	NI	NI	NI	**238,06**	NI	NI	NI	NI

Tr (min): Retention time, P (%): Percentage presence, NI: Not identified, Compound: Compound identified (if applicable), Formula: Chemical formula, M (g/mol): Molecular weight.

Quantification of Total Phenolic Compounds, Total Flavonoids, and Total Condensed Tannins

Quantification of Total Phenolic Compounds

The levels of total phenolic compounds in the hydro-ethanolic extracts from the leaves and stem barks of *Z. zanthoxyloides* are presented in Table 4. The amount of total phenolic compounds is approximately twice as high in the leaves compared to the stem barks.

Table 4: Total Phenolic Content in the Stem Barks and Leaves of *Z. zanthoxyloides.*

Organ	Total Phenolic Content (mg GAE/g DM)
ZZT	294,103 ± 8,936
ZZF	530, 575 ± 2,341

Quantification of Total Flavonoids

The total flavonoid content in the hydro-ethanolic extracts of the leaves and stem barks of *Z. zanthoxyloides* was determined. The results, shown in Table 5, indicate that the total flavonoid content is higher in the leaves than in the stem barks.

Table 5: Total Flavonoid Content in the Stem Barks and Leaves of *Z. zanthoxyloides.*

Organ	Total Flavonoid Content (mg QE/g DM)
ZZT	7,48 ± 0,30
ZZF	13,18 ± 0,96

Quantification of Total Condensed Tannins

The total condensed tannin content in the hydro-ethanolic extracts of the leaves and stem barks of *Z. zanthoxyloides* is presented in Table 6. The results indicate that the concentration of condensed tannins is significantly higher (9 times more) in the leaves compared to the stem barks.

Table 6: Total Condensed Tannin Content in the Stem Barks and Leaves of *Z. zanthoxyloides.*

Organ	Total Condensed Tannin Content (mg CE/g DM)
ZZT	5,31 ± 0,48
ZZF	46,75 ± 3,38

Antioxidant Activity

Antioxidant Activity in Test Tubes

After adding the ethanolic solution of DPPH to the hydro-ethanolic extracts of the leaves and stem barks of *Z. zanthoxyloides*, a yellow coloration appeared after a few minutes of

incubation, indicating a positive reaction. This qualitative observation confirms that the leaves and stem barks of *Z. zanthoxyloides* possess antioxidant potential against the DPPH radical.

Antioxidant Activity by Spectrophotometry

The antioxidant activity was then quantified by spectrophotometry, a more precise method based on the same principle as the test tube analysis, which is the reduction of the DPPH radical. This approach allowed for the determination of inhibition percentages and IC50 values for both plant extracts as well as for vitamin C.

Inhibition Percentages of the Hydro-Ethanolic Extracts from the Leaves and Stem Barks of *Z. zanthoxyloides* and Vitamin C

The inhibition percentages of the hydro-ethanolic extracts from the leaves and stem barks of *Z. zanthoxyloides* and those of vitamin C were determined. The various values obtained are represented in Figure 2. The inhibition percentages range from 43.984±0.000% to 99.181±0.075% for the two plant extracts, compared to percentages ranging from 95.213±0.388% to 99.784±0.075% for vitamin C at the same concentrations (2 to 0.0625 mg/mL).

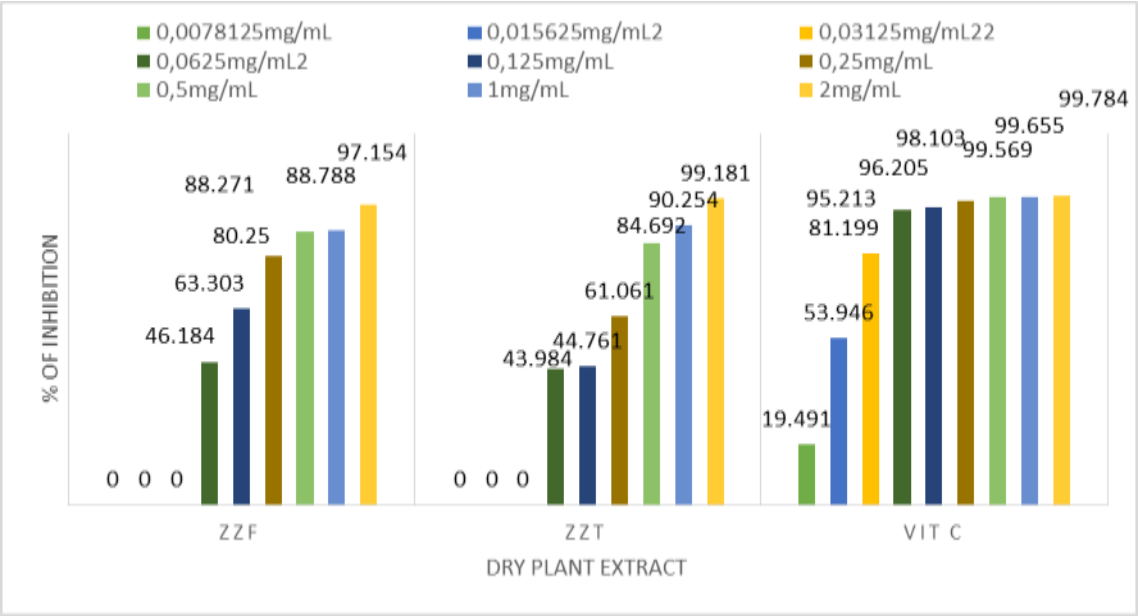

Figure 2: Inhibition of DPPH by hydroethanolic extracts of leaves and stem bark of *Z. zanthoxyloides* and Vitamin C.

Inhibitory Concentrations at 50% (IC50) of the hydroethanolic extracts of leaves and stem bark of *Z. zanthoxyloides* and Vitamin C

The inhibitory concentrations at 50% (IC50) of various plant extracts and Vitamin C were measured to evaluate their antioxidant capacity. This measurement allows for the comparison of the antioxidant efficiency of the extracts: a lower IC50 indicates greater antioxidant activity. The IC50 values, obtained after processing the data using EXCEL 2021 (version 16.0), are presented in Table 7.

Table 7: 50% inhibitory concentration of hydroethanolic extracts of leaves and stem bark of *Z. zanthoxyloides* and Vitamin C.

Extractss	Linear Equations	CI_{50} (mg/mL)
Vit C	Y= 4410,2 X – 14,964	**0,0147**
ZZF	Y= 273,9 X + 29,065	**0,076**
ZZT	Y= 130,4 X + 28,461	**0,165**

DISCUSSION

The phytochemical study of the leaves and stem bark of *Zanthoxylum zanthoxyloides* began with an extraction by maceration in a binary mixture of ethanol and water (49/21), applied to the powders of these plant organs. This extraction method is known for its ability to extract a significant amount of phytocompounds, as demonstrated by Ouattara[12] and Kabran.[13] Research conducted by Mahmoudi and collaborators[1] has also confirmed that maceration in ethanol promotes the extraction of phytocompounds. Additionally, the presence of water enhances the permeability of plant tissues and facilitates mass diffusion during the extraction step.[14-16] In this study, the leaves of *Z. zanthoxyloides* showed a higher extraction yield than the stem bark, with respective yields of 26.67±2.93% and 24.2±01.06%. This suggests that the leaves contain a greater quantity of extractable compounds through maceration in the ethanol/water solvent mixture.

The phytochemical screening revealed the presence of tannins, flavonoids, coumarins, sterols, terpenes, volatile oils, and reducing sugars in the leaves and stem bark of *Z. zanthoxyloides*. Anthocyanins were only detected in the leaves, while compounds such as free quinones, anthraquinones, alkaloids, and saponins were absent in both organs. These results are consistent with findings from other researchers, such as Bagayoko[17] and Bossokpi[18], who also showed that the leaves are rich in essential oils containing dipentene, linalool, methylnonylketone, and coumarins (bergapten). Bagayoko[17] also identified gums in the stems containing reducing sugars like arabinose, galactose, and 4-methylglucuronic acid. The

presence of these phytocompounds could explain the traditional medicinal use of *Z. zanthoxyloides* for treating various ailments, including dental caries, toothaches, onchocerciasis, urticaria, lacrimation, psychological disorders, migraines, neuralgia[19], gonorrhea, colic, diarrhea, gastritis, hypertension, ulcers, syphilitic chancres, scabies[20], rheumatism, arthritic pain, hemorrhoids, leucorrhea, and sickle cell disease.[21] Several studies have also highlighted the pharmacological properties of this plant. In Nigeria, research has shown that the plant prevents hemolysis of red blood cells.[22] These studies have also demonstrated the antimicrobial, anticancer, antiviral[23,24], anti-inflammatory, antioxidant[18, 24], antifungal, larvicidal, and molluscicidal properties of this plant.[24] Additionally, the biological properties of various phytocompounds present in the leaves and stem bark of *Z. zanthoxyloides* are widely recognized.[25] Polyphenols, such as flavonoids, tannins, coumarins, and anthocyanins, possess anti-inflammatory, antiparasitic, antimicrobial[26], antibacterial, antiviral[27], hemostatic[28], vasoconstrictive[29], and antioxidant properties.[26,30] Terpenes and sterols have analgesic and anti-inflammatory properties.[31] Essential oils are widely used in the food, pharmaceutical, and cosmetic industries for their benefits[32], while reducing sugars have a hyperglycemic effect.[33]

The search and identification of molecules in the hydroethanolic extracts of the leaves and stem bark of *Z. zanthoxyloides* were carried out using UPLC-MS, employing certain standards. This study identified the five major compounds in each extract, although only one compound was found in both extracts. This is a flavonol with the formula $C_{15}H_{10}O_3$ and a molecular mass of 238.06. It appears at a retention time of 0.78 min on the chromatogram of the stem bark, with a percentage presence of 18.98%. Flavonols, like most flavonoids, have multiple beneficial biological properties and are particularly recognized as powerful antioxidants.[34]

The total phenolic compounds, total flavonoids, and total condensed tannins were quantified by spectrophotometry to assess their presence in the hydroethanolic extracts of the leaves and stem bark of *Z. zanthoxyloides*. The results showed that the leaves contain approximately 1.8 times more total phenolic compounds than the stem bark. The uneven distribution of phenolic compounds in different parts of a plant has been reported by several authors, and this disparity can be explained by the action of biogenetic or environmental factors.[35,36] The total flavonoid content is also higher in the leaves, with a total flavonoid content approximately 1.8 times higher than that of the stem bark. The synthesis of flavonoids by plants is

stimulated by sun exposure[37], explaining the higher concentration in the leaves, as the aerial parts are more exposed. Condensed tannins are also more prevalent in the leaves, with a content approximately 8.8 times higher than in the stem bark. These results suggest that the leaves of *Z. zanthoxyloides* concentrate more total phenolic compounds, total flavonoids, and total condensed tannins than the stem bark.

The antioxidant activity of the hydroethanolic extracts of the leaves and stem bark was evaluated using both tube and spectrophotometric assays. The tube test showed antioxidant activity for both extracts. This activity could be partly explained by the presence of the different secondary metabolites detected in these two organs during the phytochemical screening and UPLC-MS analysis.[26, 27, 29, 38] The quantitative spectrophotometric analysis allowed for a comparison of the antioxidant potential of the two studied plant organs with that of Vitamin C. Thus, Vitamin C, the reference molecule with an IC50 of 0.0147 mg/mL, is more active than the two studied plant extracts. However, both plant organs exhibit notable antioxidant activity against the DPPH radical. The leaves of *Z. zanthoxyloides*, with an IC50 of 0.076 mg/mL, demonstrated greater antioxidant activity than the stem bark (0.165 mg/mL), more than twice as much. This significant difference can be explained by the higher levels of phenolic compounds, flavonoids, and condensed tannins in the leaves compared to the stem bark of *Z. zanthoxyloides*. Indeed, several studies have highlighted the potent antioxidant actions of these different secondary metabolites.[26,30] The antioxidant activity observed in the leaves and stem bark of *Z. zanthoxyloides* could explain the use of this plant in the treatment of diseases related to oxidative stress.

CONCLUSION

This study highlights the therapeutic potential of *Zanthoxylum zanthoxyloides*, a medicinal plant from northern Côte d'Ivoire, due to its phytochemical and antioxidant properties. The results reveal the presence of tannins, flavonoids, coumarins, sterols, terpenes, volatile oils, and reducing sugars in the leaves and stem bark of *Z. zanthoxyloides*. Anthocyanins were detected in the leaves, and flavonols in the stem bark. There is also a notable concentration of bioactive phytocompounds such as phenolic compounds, flavonoids, and condensed tannins in these organs, particularly in the leaves. Moreover, the leaves exhibit a stronger antioxidant activity than the stem bark. This finding could justify the traditional use of this plant for treating diseases related to oxidative stress. However, to fully optimize the use of *Z. zanthoxyloides*, further research is needed to assess the potential toxicity of its organs and to

explore its antioxidant activity further using complementary methods. In parallel, the development of a phytomedicine derived from this plant could offer a natural and effective therapeutic solution, thereby enriching the medicinal arsenal available to combat oxidative stress-related conditions.

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