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LC-MS METABOLIC PROFILE OF THE HYDRO-ETHANOLIC EXTRACT OF *ZANTHOXYLUM ZANTHOXYLOIDES* STEM BARK HARVESTED IN KORHOGO (IVORY COAST)]

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ABSTRACT

The traditional uses of *Zanthoxylum zanthoxyloides* in West Africa have sparked increased interest in the chemical characterization of its plant extracts with a view to their pharmacological exploitation. This study presents the metabolic profile obtained by liquid chromatography coupled with mass spectrometry (LC-MS) of the hydro-ethanolic extract (70/30: v/v) of the bark of *Zanthoxylum zanthoxyloides* stems harvested in Korhogo (Ivory Coast). Extraction, carried out by maceration for 24 hours, yielded an extract rich in polar and semi-polar metabolites. LC-MS analysis performed in positive mode revealed twenty-one (21) compounds, including flavonoids, coumarins, phenylpropanoid amides, lignans, and alkaloids. In negative mode, nine (09) metabolites were identified, mainly glycosides, fatty acids, and phenolic derivatives. The results reveal a diverse metabolic richness and suggest compounds that may exert antioxidant, vasculoprotective, hepatoprotective, analgesic, antiparasitic, anticancer or anti-inflammatory

activities. These data provide a reliable basis for future studies on the isolation, absolute quantification and targeted biological evaluation of molecules of interest.

KEYWORDS: *Zanthoxylum zanthoxyloides*, LC-MS, secondary metabolites, stem bark, hydro-ethanolic extraction, Ivory Coast.

INTRODUCTION

Traditional African pharmacopoeia makes extensive use of plants to treat various diseases, particularly infectious, inflammatory, and metabolic disorders.[1] Zanthoxylum zanthoxyloides is a plant of the Rutaceae family that is traditionally used for its antimalarial, analgesic, healing and antioxidant properties.[2,3,4] However, phytometabolic studies on stem bark are rare, as most research has focused on the roots, fruits or root bark[5,6] In addition, a preliminary study was conducted on the phytochemical screening of hydro-ethanolic extracts of Zanthoxylum zanthoxyloides from Korhogo (Ivory Coast) using tube characterization tests by Bamba and colleagues.[7] The results showed the presence of tannins, flavonoids, coumarins, alkaloids, sterols, terpenes, volatile oils and reducing sugars, but not free quinones, anthraquinones, saponins or anthocyanins. Following on from this work, other more advanced analytical techniques such as liquid chromatography coupled with mass spectrometry (LC-MS) were used. LC-MS is a state-of-the-art approach for the accurate identification and quantification of metabolites, while tube screening is a first-level approach limited to global qualitative analysis.[8, 9, 10]

Modern mass spectrometry techniques coupled with liquid chromatography can detect a wide range of semi-polar to polar metabolites, including flavonoids, glycosides, alkaloids, coumarins and oxylipins.[9,10,11,12]

This study aims to qualitatively characterize the metabolites present in the hydro-ethanolic extract of Z. zanthoxyloides stem bark harvested in Korhogo using LC-MS in positive and negative modes. It also aims to interpret the chemical diversity observed in relation to traditional uses and the phytometabolic literature of the genus Zanthoxylum.

MATERIAL

Plant material

The plant material consists of the bark of *Zanthoxylum zanthoxyloides* stems. The plant was harvested in February 2023 in the city of Korhogo (9° 27' 28'‘ North, 5° 37’ 46" West),

specifically in the botanical garden of Peleforo GON COULIBALY University in Korhogo. The plant was authenticated by botanists from the university. The bark of the stems was first dried for seven days away from sunlight in a room at room temperature. It was then crushed in a mortar and sieved to obtain a fine powder, which was used to prepare the extract for testing.

Laboratory equipment and solvents

The equipment used includes standard laboratory glassware, a precision electronic balance (DENVER INSTRUMENT SI-234), a thermostatic oven (Memmert) and liquid chromatography coupled with a Thermo Scientific Orbitrap Exploris 480 high-resolution mass spectrometer. The solvents used were distilled (water, 96% ethanol, pure methanol, and acetonitrile).

METHODS

Extraction

A mass of 5 g of Zanthoxylum zanthoxyloides stem bark powder was macerated in 70 mL of a binary ethanol/water mixture (70/30 : V/V) for 24 hours. After filtration, the filtrate was stored for 24 hours in a refrigerator at 4°C for the precipitation of lipophilic compounds. After decanting, the hydro-ethanolic extract obtained was placed in an oven at 50°C for 2 days to provide the dry hydro-ethanolic extract of Zanthoxylum zanthoxyloides stem bark. This dry hydro-ethanolic extract was used for LC-MS analysis.

LC-MS analysis

The sample was analyzed in nanoLCMS (nLCMS) mode on a Thermo Scientific Orbitrap Exploris 480 spectrometer in positive and negative electrospray modes. The dry hydro-ethanolic extract from the bark of Zanthoxylum zanthoxyloides stems was solubilized in a mixture of HPLC-grade H2O/CH3OH (95/5) solvents. The analyses were performed on 500 ng of material deposited on a PepMap Neo C18 pre-column, 5 µm, 300 µm x 5 mm / EASY-Spray PepMap Neo column 1500 bars, 75 µm x 150 mm, C18, 2 µm, 100 Å. LC-MS analyses were performed on a technical duplicate. LC-MS/MS analyses in positive and negative modes were performed in a single 40-minute run with the following instrumental parameters and gradient: CRMPO-PJ-P-480k-40min-100-1500-2000V-SL75-Gradient 2 - Trap and Elute – BackFlush.

The various acquisitions were processed using Compound Discoverer 3.3 SP3 (3.3.3.200) software according to the following workflow:
Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mzLogic.cdProcessingWF_AMBEU_241113.

The complete results were then filtered using the following seven (7) parameters to produce more easily usable files: the peak is not present in the blank, the chromatographic peak area is greater than 108, MS/MS acquisition was performed, at least one occurrence is present in the mzCloud database, at least one occurrence is present in the ChemSpider database, the experimental error is less than +/- 2 ppm, and the occurrence(s) in the mzCloud database are greater than 75%.

RESULTS

LC-MS analyses of the hydro-ethanolic extract of *Z. zanthoxyloides* stem bark enabled the qualitative identification of a total of thirty (30) compounds, including twenty-one (21) in positive ionization mode and nine (09) in negative ionization mode.

LC-MS analysis of the extract in positive electrospray mode

The chromatographic profile of the extract from the stems of *Zanthoxylum zanthoxyloides* in positive mode is shown in Figure 1.

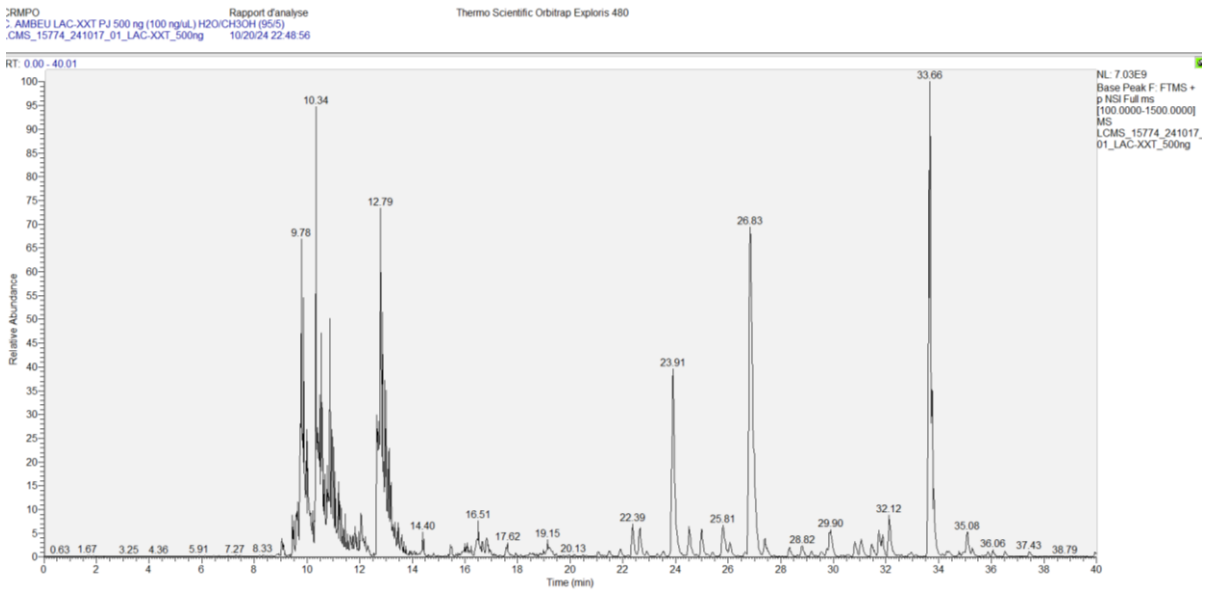


Figure 1: Chromatographic profile of *Z. zanthoxyloides* stem bark extract in positive mode.

The twenty-five (21) compounds detected by positive mode in the extract of Z. zanthoxyloides stem bark include conjugated amides ((2E,4E)-N-(2-methylpropyl)deca-2,4-dienamide; various acylamides), flavonoids and methoxylated derivatives (hesperidine, 3′,5,7-trihydroxy-4′-methoxyflavanone, etc.), coumarins (7-hydroxy-6-methoxy-2H-chromen-2-one, scoparone), glycosides (various flavonoid glycosides or cyclohexane glycosides), lignans (matairesinol), oxylipins, acids (12-oxo phytodienoic acid) and alkaloids ((+)-Evodiamine, complex polycyclic structures, NP-000587, etc.) (Table 1). Table 1 shows the nomenclature, structure, retention time, molecular formula, recalculated exact mass, and mzCloud Best Match values for each of the 21 identified compounds.

Table 1: Physicochemical parameters of molecules identified in positive mode.

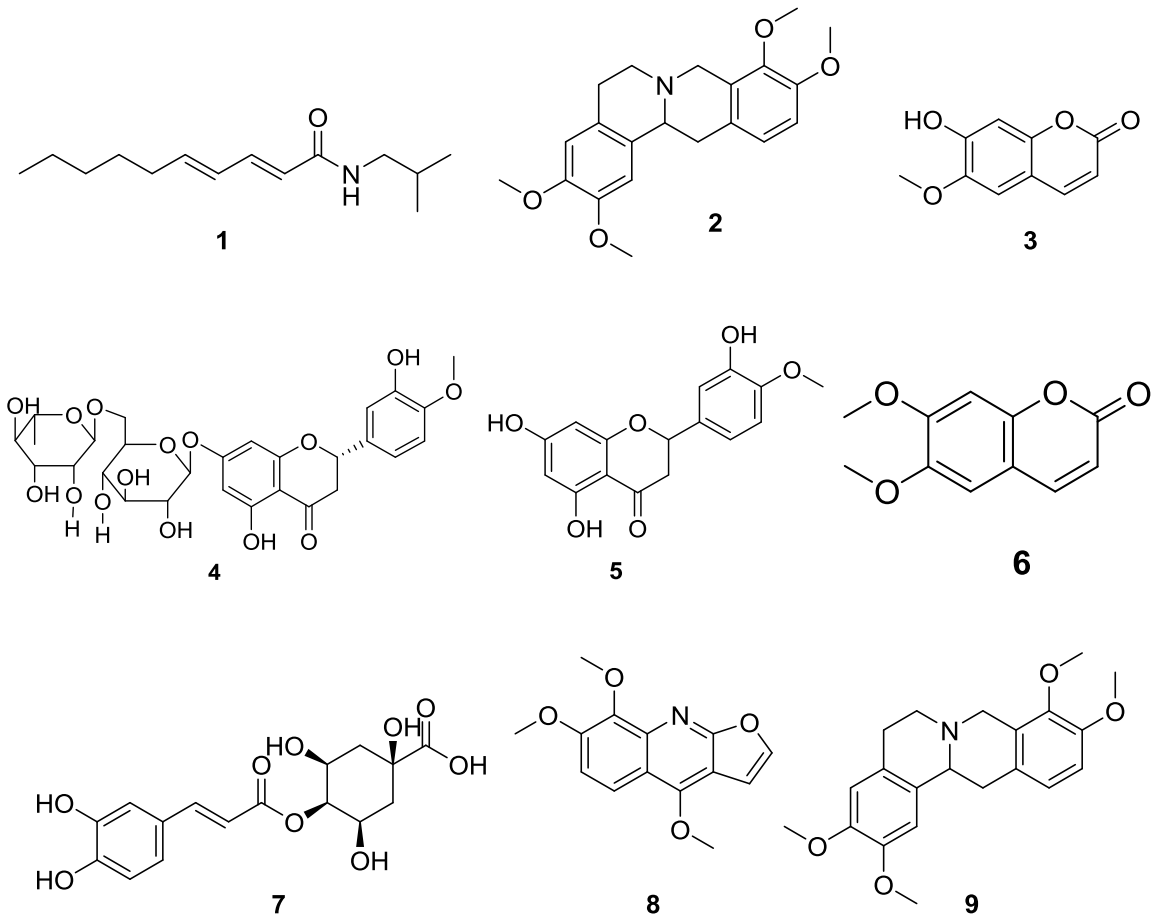
Molecule	Nomenclature of compounds	Retention time (min)	Molecular formula	Recalculated molar mass *	mzCloud Best Match**
1	(2E,4E)-N-(2-methylpropyl)deca-2,4-dienamide	34.017	$C_{14}H_{25}NO$	223.19338	96.9
2	3,4,10,11-tetramethoxy7,8,12b,13-tetrahydro-5H-6- azatetraphene	12.768	$C_{21}H_{25}NO_4$	355.17803	94.1
3	7-hydroxy-6-methoxy-2H-chromen-2-one	16.478	$C_{10}H_8O_4$	192.04223	90.3
4	Hesperidin	17.186	$C_{28}H_{34}O_{15}$	610.18952	98.4
5	3',5,7-Trihydroxy-4'- methoxyflavanone	17.182	$C_{16}H_{14}O_6$	302.07899	99.5
6	Scoparone	19.515	$C_{11}H_{10}O_4$	206.05788	90.1
7	(1R,3R,4S,5S)-4-{[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,3,5-trihydroxycyclohexane-1- carboxylic acid	12.338	$C_{16}H_{18}O_9$	354.09508	98.9
8	4,7,8-trimethoxyfuro[2,3- b]quinoline	24.191	$C_{14}H_{13}NO_4$	259.08443	99.0
9	3,4,10,11-tetramethoxy-7,8,12b,13-tetrahydro-5H-6-azatetraphene	14.717	$C_{21}H_{25}NO_4$	355.17843	92.6
10	6,7,8-trimethoxy-2H-chromen-2-one	21.837	$C_{12}H_{12}O_5$	236.06851	96.2
11	(2E)-3-(4-Hydroxyphenyl)-N-[2- (4-hydroxyphenyl)ethyl]acrylamide	20.113	$C_{17}H_{17}NO_3$	283.12085	99.6
12	Matairesinol	23.942	$C_{20}H_{22}O_6$	358.14167	98.7
13	(3R,5R)-1,3,5-Trihydroxy-4- {[(2E)-3-(4-hydroxy-3- methoxyphenyl)-2-propenoyl]oxy} cyclohexanecarboxylic acid	14.729	$C_{17}H_{20}O_9$	368.11085	97.3
14	3-hydroxy-7,8,14-trimethoxy-17- methyl-12,16,21- trioxapentacyclo]henicosa-2, 4(13),6,8,10,14-hexaen-5-one	27.373	$C_{22}H_{22}O_8$	414.13147	75.1
15	(3aR,7aS,8S,9aR)-5,8-dimethyl3-methylidene2H,3H,3aH,4H,6H,7H,7aH,8H, 9H,9aH-azuleno[6,5-b]furan-2,6- dione	10.757	$C_{15}H_{18}O_3$	246.12567	86.6
16	12-Oxo phytodienoic acid	33.805	$C_{18}H_{28}O_3$	292.20385	92.1
17	4-methoxy-6-(prop-2-en-1-yl)-2H-1,3-	14.628	$C_{11}H_{12}O_3$	192.07866 8	84.6

	benzodioxole				
18	α-Eleostearic acid	35.119	$C_{18}H_{30}O_2$	278.22458	96.3
19	(+)-Evodiamine	30.487	$C_{19}H_{17}N_3O$	303.13717	95.3
20	N-[(4-hydroxy-3- methoxyphenyl)methyl]-8- methylnonanamide	21.337	$C_{18}H_{29}NO_3$	307.21479	99.1
21	NP-000587	14.064	$C_{16}H_{18}O_8$	338.10025	97.7

*The recalculated molar mass (calc. MW) indicated in Compound Discoverer reports actually corresponds to the exact mass recalculated to an experimental error (e.g., for compound 4: $C_{28}H_{34}O_{15}$ calc. m/z: 610.18952; theoretical m/z: 610.18922).

**mzCloud Best Match corresponds to a similarity score between the fragmentation spectrum (MS/MS) of the analyzed compound and the reference spectra in the mzCloud database. In other words, mzCloud is a confidence score or consistency score between the experimental isotopic profile and the theoretical one. The closer the mzCloud value is to 100, the more confidently the compound has been identified.

The different structures of the molecules identified in positive mode in the extract of *Z. zanthoxyloides* stem bark are shown in Figure 2.



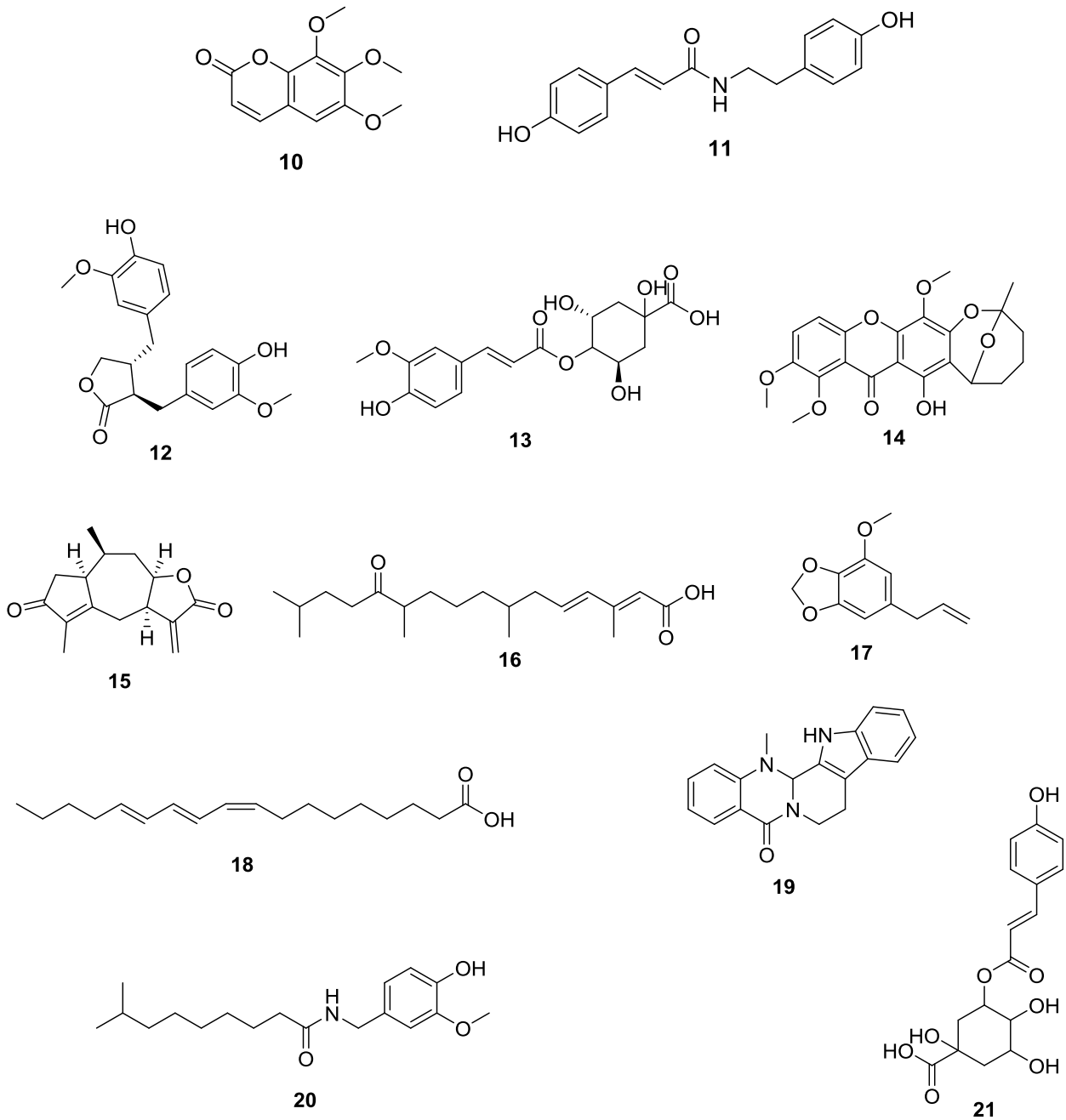


Figure 2: Structures of phytochemicals identified in positive mode.

LC-MS analysis of the extract in negative electrospray mode

The chromatographic profile of the extract from the stems of *Zanthoxylum zanthoxyloides* in negative mode is shown in Figure 3.

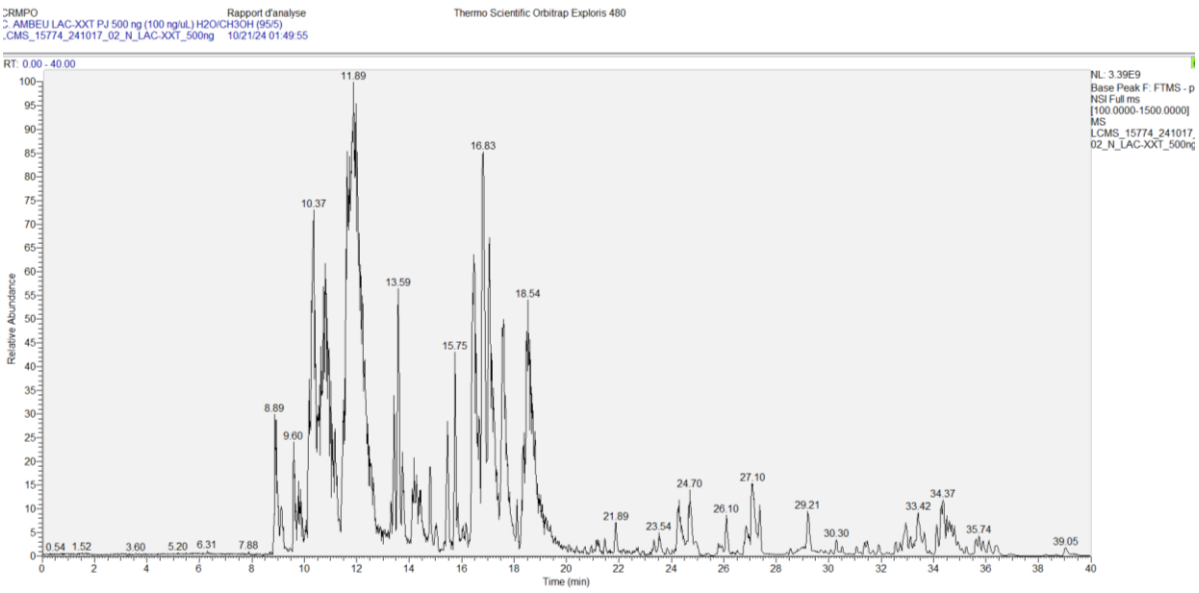


Figure 3: Chromatographic profile of *Z. zanthoxyloides* stem bark extract in negative mode.

The nine (09) compounds identified in negative mode in the extract of *Z. zanthoxyloides* stem bark mainly belong to flavonoids (3',5,7-trihydroxy-4'-methoxyflavanone), oxylipins (±9(10)-EpOME, ±9-HpODE, ±12 (13)-DiHOME), acids (stearic acid), and lignans ((+)-syringaresinol) (Table 2). Table 2 shows the nomenclature, structure, retention time, molecular formula, recalculated exact mass and mzCloud Best Match values for each of the 09 compounds identified.

Table 2: Physicochemical parameters of molecules identified in negative mode.

Molecule	Nomenclature of compounds	Retention time (min)	Molecular formula	Recalculated molar mass*	mzCloud Best Match**
1	2-(4-Hydroxyphenyl)ethyl 6-O-[(2R,3R,4R)-3,4-dihydroxy-4-(hydroxymethyl)tetrahydro-2-furanyl]-beta-D-glucopyranoside	10.507	$C_{19}H_{28}O_{11}$	432.16235	91.4
2	(±)9(10)-EpOME	34.666	$C_{18}H_{32}O_3$	296.23489	83.0
3	3',5,7-Trihydroxy-4'-methoxyflavanone	17.103	$C_{16}H_{14}O_6$	302.07900	98.2
4	NP-011548	36.048	$C_{18}H_{34}O_3$	298.25052	92.5
5	(+)-Syringaresinol	21.361	$C_{22}H_{26}O_8$	418.16195	95.2
6	3-[4-(beta-D-Glucopyranosyloxy)-6-methoxy1-benzofuran-5-yl]propanoic acid	15.987	$C_{18}H_{22}O_{10}$	398.12052	88.0
7	(±)9-HpODE	31.010	$C_{18}H_{32}O_4$	312.22994	95.4

8	Stearic Acid	39.102	$C_{18}H_{36}O_2$	284.27150	96.8
9	(±)12(13)-DiHOME	31.514	$C_{18}H_{34}O_4$	314.24562	80.9

The different structures of the molecules identified in negative mode in the extract of *Z. zanthoxyloides* stem bark are shown in Figure 4.

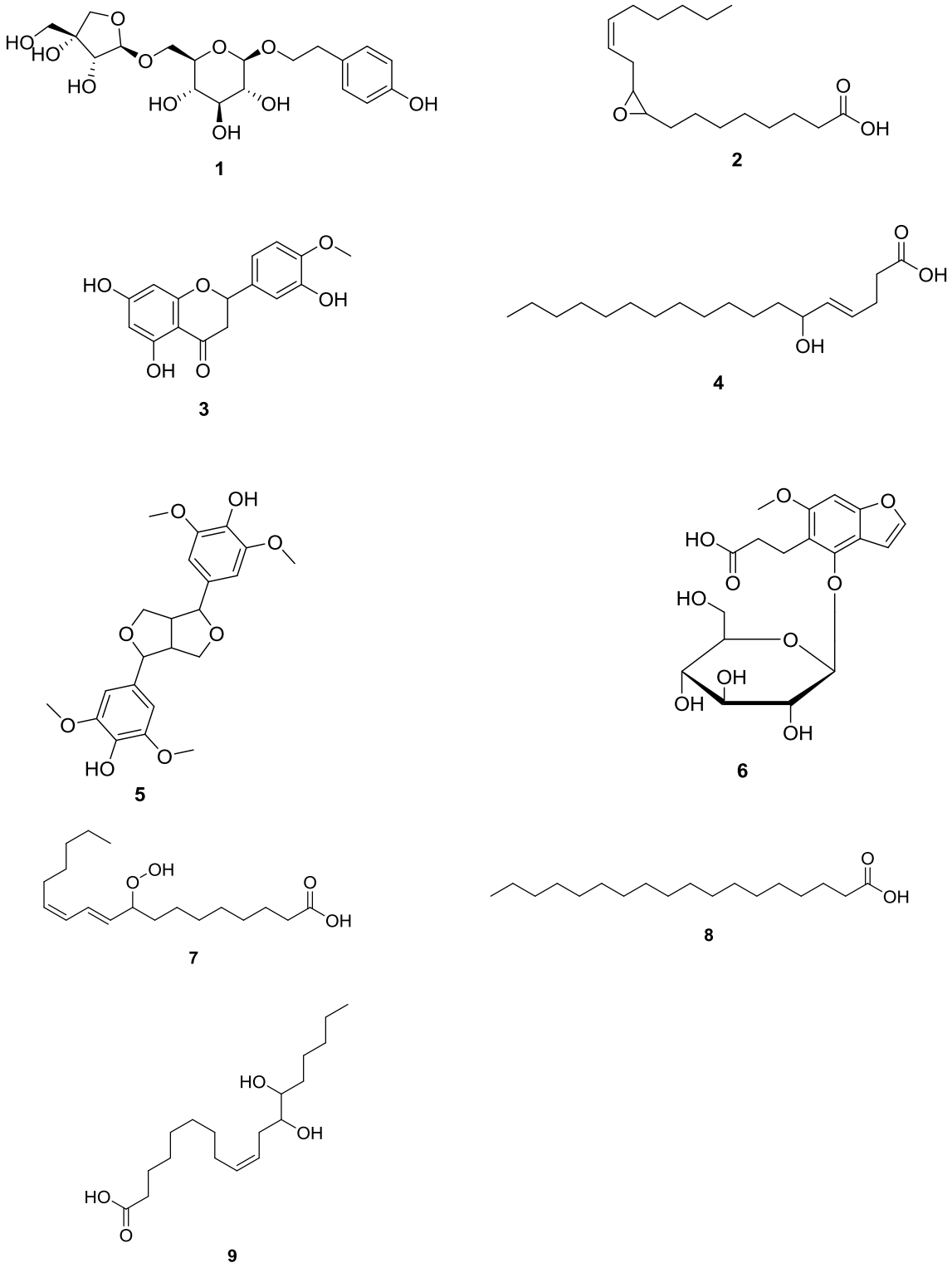


Figure 4: Structures of phytochemicals identified in negative mode.

DISCUSSION

This study is part of a research initiative aimed at documenting the metabolic profiles of African medicinal species in order to link traditional activity and chemical composition.[1, 13, 14, 15] Although *Z. zanthoxyloides* has already been studied in terms of its fruits and root bark, few LC-MS analyses have focused on its stems, and even fewer have used hydro-ethanolic extraction.

The ethanol/water mixture is known to extract polar and semi-polar compounds effectively.[11] Maceration for 24 hours at room temperature prevents thermal degradation and preserves sensitive metabolites such as oxylipins, which can break down if the sample is heated or exposed to oxygen.[16,17] This strategy has made it possible to identify a representative set of compounds, covering both water-soluble and fat-soluble molecules.

LC-MS analysis identified twenty-one (21) compounds in positive mode and nine (09) compounds in negative mode. Positive mode revealed basic and neutral compounds such as alkaloids ((+)-evodiamine), phenylpropanoid amides and methoxylated coumarins. Negative mode, on the other hand, revealed acidic or glycosylated metabolites (syringaresinol, hydroxylated fatty acids). This complementarity of ionization modes provides a comprehensive metabolic spectrum and highlights the value of a dual approach for studying complex plant matrices.[18]

The choice of ionization mode in electrospray ionization (ESI) mass spectrometry strongly influences the detection of metabolites according to their chemical nature. As a general rule, positive mode excels at detecting basic or neutral compounds with amine or protonatable heterocyclic functions, while negative mode is more suitable for acidic or highly polar molecules, particularly phenolic acids and glycosides.[19] This complementarity reinforces the relevance of using both modes to maximize the metabolic coverage of complex plant extracts such as those from *Z. zanthoxyloides.*

The compounds identified by these two modes of analysis mainly belong to five (05) families:

- Flavonoids (3′,5,7-trihydroxy-4′-methoxyflavanone, hesperidin), which are known for their antioxidant and vasculoprotective properties.[20] Hesperidin has particular antioxidant, anti-inflammatory, and cytotoxic effects.[21]

- Coumarins (scoparone, 7-hydroxy-6-methoxycoumarin), which have anti-inflammatory and hepatoprotective properties. [22, 23]
- Amides and alkaloids ((2E,4E)-N-(2-methylpropyl)deca-2,4-dienamide, (+)-evodiamine) are responsible for analgesic and antiparasitic effects.[24]
- Lignans (materesinol, syringaresinol) are associated with antioxidant and anticancer properties.[25]
- Oxygenated fatty acids or oxylipins ((±)9(10)-EpOME, (±)12(13)-DiHOME) are involved in anti-inflammatory mechanisms.[26] In addition, ±12(13)-DiHOME is an oxidation of fatty acids known for its effects on oxidative stress signaling.[27]

The metabolic diversity observed supports the biological activities reported for *Z. zanthoxyloides*, particularly its antioxidant, anti-inflammatory, and antiparasitic effects. Flavonoids and lignans contribute to the neutralization of free radicals, while amides and alkaloids may explain the analgesic and antiparasitic effects of the species.[28, 29]

The co-presence of flavonoids, coumarins, lignans, and oxylipins confirms the typical chemical classes of *Zanthoxylum* species.[30] For example, the identification of hesperidin in this extract is consistent with similar results in Citrus and supports the presence of glycosylated flavanones in Rutaceae.[31]

LC-MS analyses performed on the roots and leaves of *Z. zanthoxyloides* revealed similar profiles dominated by amides and flavonoids.[25, 32] However, stem bark exhibits greater diversity in lignans and coumarins, suggesting metabolic specificity related to the protective function of this organ. Geographic and ecological variations may also influence the biosynthesis of these metabolites.[33]

LC-MS analysis in positive and negative modes, combined with database comparison and MS/MS fragment analysis, enabled reliable identification of the presumed compounds. Here, structural identification is more abundant than in our previous work on LC-MS analysis of *Anogeissus leiocarpus*[34] and *Cassia sieberiana*.[35] This study paves the way for further work on the isolation, quantification, and biological evaluation of the identified compounds.

CONCLUSION

This study qualitatively characterized the hydro-ethanolic extract of *Zanthoxylum zanthoxyloides* stem bark harvested in Korhogo (Ivory Coast), revealing thirty (30)

compounds distributed between twenty-one (21) in positive ionization mode and nine (09) in negative mode. The dominant chemical classes include flavonoids (glycosylated and methoxylated), alkaloids, phenolic acids, coumarins, oxylipins, and lignans. The complementarity of the LC-MS ionization modes provided broader metabolic coverage than is often reported for a single mode. Several identified metabolites, such as hesperidin, scoparone, (+)-evodiamine, syringaresinol and certain oxylipins, have well-documented biological activities, which warrants further studies on isolation, absolute quantification and targeted biological activity. These results enrich the phytochemical database of *Zanthoxylum zanthoxyloides* and reinforce its potential as a source of natural bioactive compounds.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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