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Two isoquinoline alkaloids isolated from the leaves of *Monodora tenuifolia* Benth. (Annonaceae)

Logopho Hyacinthe Ouattara [1, *], Souleymane Bamba [2], Richmond Jean-François Kablan [1, 3], Ahmont Landry Claude Kablan [1, 3], Ouayogode Aminata Akoubet [4], Obiang Cédric Sima [5] and N'dri Marcelline Adiko [4]

[1] *UPR Chemistry, Department of Mathematics-Physics-Chemistry, Faculty of Biological Sciences, Peleforo GON COULIBALY University, BP 1328 Korhogo, Ivory Coast.*
[2] *Laboratory of Environmental Sciences and Technologies, Faculty of Environment, Jean Lorougnon GUEDE University, BP 150 Daloa, Ivory Coast.*
[3] *COVACHIM-M2E EA 3592 Laboratory, Fouillole Campus, Faculty of SEN, Department of Chemistry, Pointe-à-Pitre Cedex.*
[4] *Laboratory of Medicinal Sciences, Analytical Sciences and Public Health, Department of Pharmaceutical Sciences, Teaching Unit 1 (Pharmacognosy, Botany, Plant Biology, Cryptogamy), UFR SPB, UFHB, Cocody (Côte d'Ivoire).*
[5] *Laboratory for Research in Biochemistry (LAREBIO), Faculty of Sciences, University of Science and Technology of Masuku, Franceville, Gabon.*

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Abstract

Monodora tenuifolia is a species of plant in the Annonaceae family native to Africa. The Monodora genus contains isoquinoline alkaloids, compounds known for their many therapeutic properties. These are chemotaxonomic markers of the genus. This study describes the isolation and identification of two isoquinoline alkaloids from the leaves of *Monodora tenuifolia* Benth. (Annonaceae). Structural analysis was performed using one-dimensional nuclear magnetic resonance (^{1}H and ^{13}C NMR) and two-dimensional COSY, HSQC, HMBC, and NOESY techniques. The spectra obtained enabled the compounds to be formally identified as (-)-N-methylarmepavine, a benzylisoquinoline, and (+)-listeferine, an oxoaporphine. These results constitute the first evidence of these two alkaloids in the leaves of *M. tenuifolia.* The study also highlights the correlations between these structures and their known pharmacological properties, such as the antioxidant, antimicrobial, and neuroprotective activities reported in the literature. These data contribute to the enrichment of the chemical profile of the *Monodora* genus and provide a solid foundation for future biological investigations.

Keywords: *Monodora tenuifolia;* Isoquinoline alkaloids; NMR; Ivory Coast

1. Introduction

The Annonaceae family, widely distributed in tropical and subtropical areas, is known for its richness in secondary metabolites with high biological value, including alkaloids, acetogenins, flavonoids, and terpenoid derivatives [1, 2, 3]. Among the most characteristic compounds of this family are isoquinoline alkaloids, biosynthesized from tyrosine and comprising structural subclasses such as benzylisoquinolines, aporphines, oxoaporphines, and protoberberines [4, 5].

The genus *Monodora*, belonging to the Annonaceae family, includes several species used in traditional African medicine for their anti-infectious, analgesic, and anti-inflammatory properties [6, 7]. While some species, such as *Monodora myristica*, have already been the subject of extensive phytochemical research revealing the presence of isoquinoline alkaloids [8], *Monodora tenuifolia* Benth. remains relatively understudied. The few studies available have mainly focused on crude extracts from roots or seeds, with a few articles on the leaves [9, 10].

* Corresponding author: Ouattara Logopho Hyacinthe.

Leaves, which are often underutilized in studies of natural substances, may nevertheless contain specific bioactive alkaloids, which can vary depending on the plant organ [11]. The chemical exploration of this matrix thus offers an opportunity to discover new structural compounds or to confirm the distribution of alkaloids already known in other organs or species.

In this context, the present study aims to isolate and identify isoquinoline-type alkaloids from the leaves of *Monodora tenuifolia*. Structural elucidation was mainly carried out using ^{1}H and ^{13}C NMR spectroscopy, reinforced by two-dimensional COSY (^{1}H-^{1}H), HMBC (long-range ^{1}H-^{13}C), and NOESY (^{1}H-^{1}H through space) correlations, allowing the determination of atomic connectivity, electronic environment, and spatial geometry of the protons involved.

This combined approach aims to confirm the proposed structures and contribute to the enrichment of chemical knowledge about the *Monodora* genus, while providing useful reference data for possible future pharmacological investigations.

2. Material and methods

2.1. General

The NMR spectra were recorded on a Brüker Advance-300 operating at 300 MHz, using TMS as the internal standard. Chemical shifts were expressed in d ppm and the coupling constant *J* was measured in Hertz (Hz). The one-dimensional ^{1}H and ^{13}C spectra were acquired under standard conditions. Currently, homonuclear ^{1}H-^{1}H (COSY, NOESY) and heteronuclear ^{1}H-^{13}C (HSQC, HMBC) correlation techniques are commonly used in the field of constitutional analysis. These techniques were recorded on a Brücker Avance-400 operating at 400 MHz. Column chromatography was performed on silica gel (Kieselgel 60, particle size 0.040-0.063 mm) and Sephadex®LH-20. TLC was performed on glass plates pre-coated with silica gel (Merck silica gel 60 F_{254}). Spots were detected by spraying with Dragendorff's reagent or 50% H_2SO_4 and phosphomolybdic acid. This operation was followed by heating. The ESIMS were obtained with an ITQ 900 spectrometer using an Agilent DB-5HT column (30 x 0.32 x 0.1). Gas chromatography was performed on a Thermo Scientific TRACE GC ULTRA instrument. HR-ESIMS were performed on a WATERS Premier TOF LCT coupled with an Alliance 2695 HPLC (Waters) and also with a Bruker microTOF-Q. IR spectra were measured on a Bruker Vector. Optical rotations were recorded on an Optical Activity PolAAr polarimeter using a sample concentration of 10 mg/ml, unless otherwise indicated.

2.2. Plant material

The plant material consists of *Monodora tenuifolia* leaves that were harvested for the first time as part of this study in August 2015 in Adiopodoumé, a town in southern Côte d'Ivoire. The plant was identified at the National Center for Floristics (CNF) at Félix HOUPHOUËT-BOIGNY University. A reference specimen is kept in the herbarium of the university's botany laboratory.

2.3. Isolation

The pulverized and air-dried leaves of *M. tenuifolia* (3000 g) were degreased three times with petroleum ether, then extracted successively with dichloromethane (CH_2Cl_2), ethyl acetate (AcOEt), and methanol (MeOH). The collected fractions were evaporated under reduced pressure to yield 9.9 g of petroleum ether extract, 9.6 g of CH_2Cl_2 extract, 7.8 g of AcOEt extract, and 4.2 g of MeOH extract. The MeOH extract was fractionated by silica gel column chromatography, eluting with hexane-methanol gradient systems. Twelve fractions (F-1 to F-12) were obtained. Fraction F-4 was purified using Sephadex® LH-20 [CH_2Cl_2 : MeOH (1:1)] and silica gel column chromatography [AcOEt:MeOH (85:15)] to yield 8.1 mg of compound 1 (Figure 1) and 6.6 g of compound 2 (Figure 2).

3. Results

Two compounds were isolated from *Monodora tenuifolia*: (-)-N-methylarmepavine (MB.4) and (+)-listiferine (MC.4).

3.1. Physicochemical characteristics and spectroscopic data of the compounds

3.1.1. Compound 1: (-)-N-methylarmepavine (MB.4)

Appearance: solid, amorphous, yellowish

[a]^{D} 23 (O) = - 320,0 ; c 6,0 g/mol, MeOH ; 589 nm, IR (film, $CHCl_3$): ν_{max} = 2920 ; 2336 ; 1260 ; 718 (cm^{-1}), Molecular formula: $C_{20}H_{26}NO_3$; Rf : 0,1 MeOH/H_2O (50:50), HR-ESI-MS : *m/z* = 328,1946 [M] $^{+}$ (calculated mass 328,1947, calculated for $C_{20}H_{26}NO_3$, mDa = 0,2), UV (nm): 221,4 ; 268,5 ; 301,6 (MeOH), SMIE : *m/z* (%) 328 $[M]^+$ (17 %), 283 $[M\text{-}H\text{-}N(CH_3)_2]^+$ (22 %), 268$[M\text{-}H\text{-}N(CH_3)_2\text{-}CH_3]^+$ (13 %), 252[M- H-$N(CH_3)_2$-CH_3 –OH]$^+$ (22 %), 237 [M - H-$N(CH_3)_2$-2CH_3 – OH]$^+$ (8 %)

3.1.2. Compound 2: (+)-listeriferin (MC.4)

Appearance: solid amorphous brown

[a]^{D} 22 (O) = + 321,0 ; c 1,0 mg/ml, MeOH ; 546 nm ; IR (film, $CHCl_3$): ν_{max} = 2964 ; 1738 ;1466 ; 1260 ; 1097 (cm^{-1}) ; Molecular formula: $C_{19}H_{21}NO_4$; Rf : 0,13 AcOEt/MeOH (50:50), ESI-MS : *m/z* = 311 $[M+H]^+$, UV (nm): 220,3 ; 281,5 ; 307,6 ; 364,4 (MeOH).

3.2. Structural elucidation of compounds

3.2.1. Compound 1

Compound MB.4 is a yellowish amorphous solid. This molecule has a molecular formula of $C_{20}H_{26}NO_3$, derived from its high-resolution mass spectrum HR-ESI-MS, showing a molecular peak at *m/z* 328,1946 $[M]^+$ (calculated mass 328.1907 for $C_{20}H_{26}NO_3$). The UV spectrum of compound MB.4 shows absorption bands at 221.4 and 268.5 nm, characteristic of benzyltetrahydroisoquinolines.

The ^1H NMR spectrum analysis shows the presence of two doublets integrating for two protons at δ_H 6.75 ppm (Jortho = 8.4 Hz); 6.87 ppm (Jortho = 8.4 Hz); 6.75 ppm (*J*ortho = 7.6 Hz) and 6.87 ppm (*J*ortho = 7.6 Hz). These protons are attributable to the protons of the C nucleus of benzyl-tetrahydroisoquinolines, monosubstituted at 13. The correlation on the COSY spectrum of the signal at δ_H 6.75 ppm (H-11) with that at δ_H 6.87 ppm (H-12) on the one hand, but also between the signal at δH 6.87 ppm (H-14) and that at δH 6.75 ppm (H-15) on the other hand, confirm these data. Furthermore, on the HSQC spectrum, the correlation between the protons at δ_H 6.87 ppm (H-12) and δ_H 6.87 ppm (H-14) with the carbons at δ_C 116.6 ppm (C-12 and C-14) indicates a possibility of ortho substitution of these protons by an oxygenated group.

HMBC spectrum analysis provides confirmation by showing a correlation between the protons at δ_H 6.75 ppm (H-15), 6.75 ppm (H-11), 6.87 ppm (H-12) and the highly unshielded quaternary carbon at δ_C 158.1 ppm (C-13). The singlets at δ_H 5.71 and 6.86 ppm, correlating respectively in HSQC with the carbons at δ_C 112.4 and 113.3 ppm, were assigned to the protons of nucleus B based on the study of the HMBC spectrum. The split doublet at δ_H 4.61 ppm, integrating for one proton, is attributable to the angular methine in α of the two N-methyl groups of ring A. Its connectivity on the COSY spectrum with the protons at δ_H 2.89 and 3.67 ppm allows the geminal protons in position 9 to be defined. The methylene groups of ring A ((H-3α; H-3β) and (H-4α; H-4β)) are visible at δ_H 3.67, 3.84, 3.24, and 3.25 ppm, respectively. The correlation between these signals on the COSY spectrum confirms these data. The intense singlets at δ_H 3.40 and 3.84 ppm on the one hand and at δH 3.17 and 3.46 ppm on the other, each integrating for three protons, are attributable respectively to two methoxyl groups and two N-methyl groups.

The ^{13}C NMR confirms our structural hypothesis. In the field of aromatic compounds, the signals at δ_C 132.5 ppm (C-11 and C-15) and 116.6 ppm (C-12 and C-14) are attributable to the other unsubstituted carbons of the C ring of a benzyltetrahydroisoquinoline.

These assignments were made possible by studying the HSQC and HMBC spectra (Table I).

The carbons of ring A of a benzyltetrahydroisoquinoline are observed at δ_C 74.3 (C-1), 55.7 (C-3), and 24.4 ppm (C-4). The carbons of the two methoxyl groups, the two N-methyl groups, and the methylene group at position 9 resonate at δ_C 55.9, 56.4, 51.4, 53.0, and 38.2 ppm, respectively. The quaternary carbons carrying an oxygenated group appear at δc 158.1 (C-13); 150.9 (C-6) and 148.3 (C-7). The other quaternary carbons are observed at δc 121.8 (C-4a), 123.1 (C-8a) and 127.1 ppm (C-10). These assignments were made based on the HMBC spectrum. Indeed, the correlation between the methoxyl protons at δ_H 3.84 ppm [OCH_3 (C-6)], the aromatic proton at δ_H 6.86 ppm (H-5) with the common carbon signal at δ_C 150.9 ppm (C-6) allows us to place these methoxyl groups in the ortho position of this aromatic proton, i.e., at C-6. The correlation between the methoxyl protons at δ_H 3.40 ppm, the aromatic proton at δ_H 5.71 ppm with the carbon at δ_C 148.3 ppm, allows us to place these methoxyl groups in the ortho position of this proton, i.e. at position 7. This is confirmed by the NOESY spectrum, which shows a correlation between the proton at position 5 and the methoxyl protons at position 6 (Table I).

On the HMBC spectrum, the long-distance 3*J* correlations observed between the signal at δ_H 6.86 ppm (H-5) and the carbon at δ_C 148.3 ppm (C-7), but also between the methylene proton at δ_H 4.61 ppm (H-1) and the carbon at δ_C 112.4 ppm (C-8), allow these different carbons and protons to be unambiguously identified. Furthermore, on the HMBC spectrum, 2*J* and 3*J* correlations are observed between the geminal methylene protons at 9 and the methine proton at δ_H 4.61 ppm (H-1) with the carbon at δ_C 127.1 ppm. The latter is attributable to the alkyl-substituted carbon (C-10) of benzyl-tetrahydroisoquinolines.

The absolute configuration R of carbon 1 of compound MB.4 is deduced from the negative sign of the optical rotation (-320). All of these spectral data (MS, IR, UV, ^{1}H NMR, and ^{13}C NMR) allow us to confirm that compound MB.4 is (-)-N-methylarmepavine (Figure 1).

Table 1 Chemical shift in ^{1}H and ^{13}C NMR (1D and 2D) of compound MB.4 (MeOD)

N° Atom	**^{13}C (δ, ppm)**	**^{1}H (δ, ppm ; m ; *J* in Hz)**	**COSY**	**HMBC**	**NOESY**
1	74,3	4,61 ; 1H ; dd (10,8 ; 3,2)	H-9α ; H-9β	C-4a ; C-8a	N-$(CH_3)_1$; H-8
3	55,7	3,67 ; 1H ; dt (12,4 ; 4,0) 3,84 ; 1H ; m	H-4a	C-1 ; C-4a	H-9α
4	24,4	3,24 ; 1H ; m 3,25 ; 1H ; m	H-3α	N-$(CH_3)_1$	-
4a	121,8	-	-	-	-
5	113,3	6,86 ; 1H ; s	-	C-4a ; C-6 ; C-7 ; C-8a	OCH_3 (6)
6	150,9	-	-	-	-
7	148,3	-	-	-	-
8	112,4	5,71 ; 1H ; s	-	C-1 ; C-4a ; C-7	H-1 ; OCH_3 (7)
8a	123,1	-	-	-	-
9α 9β	38,2 -	2,89 ; 1H ; t (12,4) 3,67 ; 1H ; dt (12,4 ; 4,0)	H-1 H-1	C-1 -	H-3 -
10	127,1	-	-	-	-
11	132,5	6,75 ; 1H ; d (8,4)	H-12	C-9 ; C-12 ; C-13	-
12	116,6	6,87 ; 1H ; d (8,4)	H-11	C-10 ; C-13	-
13	158,1	-	-	-	-
14	116,6	6,87 ; 1H ; d (7,6)	H-15	C-10 ; C-13	-
15	132,5	6,75 ; 1H ; d (7,6)	H-14	C-13 ; C-14	-
OCH_3 (6)	56,4	3,84 ; 3H ; s	-	C-6	-
OCH_3 (7)	55,9	3,40 ; 3H ; s	-	C-7	-
N-$(CH_3)_1$ N-$(CH_3)_2$	51,4 53,0	3,17 ; 3H ; s 3,46 ; 3H ; s	- -	- -	- -

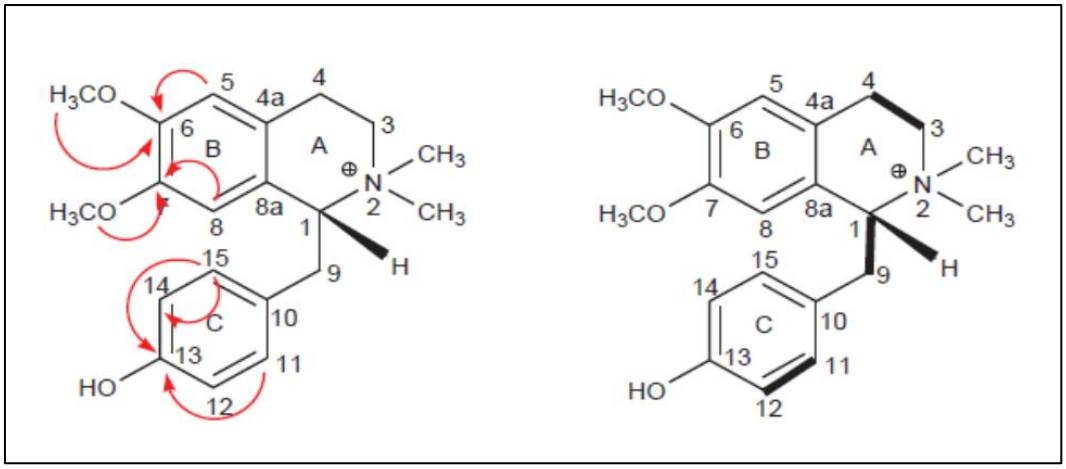

Figure 1 HMBC (⤾) and COSY (—) correlations of (-)-N-methylarmepavine (MB.4)

3.2.2. Compound 2

Compound MC.4 is isolated as a brown amorphous solid. The ESI mass spectrum shows the molecular ion peak at *m/z* 311 $[M+H]^+$, suggesting $C_{18}H_{17}NO_4$ as the molecular formula. The UV spectrum shows absorption bands at 220.3, 281.5, 307.6, and 364.4 nm. These values indicate the presence of an aporphine. This is confirmed by analysis of the ^1H NMR spectrum, which indicates the presence of a methoxyl group at δ_H 3.87 ppm and three aromatic protons resonating at δ_H 6.47; 6.79, and 7.55 ppm in the form of singlets. The proton at δ_H 7.55 ppm is attributable to the proton at position 11. This proton is shifted to a low field by the anisotropy of the D ring. We also observe the presence of methylenedioxyl groups whose protons appear at δH 5.88 and 6.00 ppm located on carbons C-2-C-3. The presence of these different groups is corroborated by the study of the ^{13}C NMR spectrum, which shows carbon signals from the methylenedioxyl group at δ_C 100.4 ppm, from the aromatic methoxyl group at δc 55.5 ppm, and from aromatic carbons at δc 126.3; 110.9, and 113.9 ppm (Table II). Analysis of the HMBC spectrum indicates a correlation between the methylene protons at δH 2.96 ppm and the carbons at δc 28.4 and 53.3 ppm. This correlation allows us to place this methylene and the angular methine in the α position of the nitrogen. The correlations between the proton at 6.47 ppm and the carbons at 144.1 and 28.4 ppm, and between the proton at 7.55 ppm and the carbons at 123.6 and 146.6 ppm, allow the aromatic protons to be placed in positions 3, 8, and 11. On the COSY spectrum, correlations are also observed between the methylene group protons (H-4α and H-5α) and the aromatic protons (H-8 and H-9). The different protons are carried by adjacent peaks. Since the rotatory power of compound MC.4 is positive (+ 321.0), the configuration of carbon 6a is *S.*

The concordance of spectral data (SM, ^1H and ^{13}C NMR) allows us to suggest that the structure of compound MC.4 is that of (+)-listeferine (Figure II).

Table II ^{1}H and ^{13}C NMR chemical shifts (1D and 2D) of compound MC.4 (MeOD)

N° Atom	^{13}C (δ, ppm)	^{1}H (δ, ppm ; m ; J in Hz)	COSY	HMBC	NOESY
1	144,1	-	-	-	-
1a	123,6	-	-	-	-
1b	125,8	-	-	-	-
2	146,7	-	-	C-1 ; C-1b ; C-2 ; C-4	-
3	106,7	6,47 ; 1H ; s	-	-	-
3a	126,3	-	-	-	-
4	28,4	2,65 ; 1H ; d (7,2) 2,96 ; 1H ; d (8,0)	H-5α H-5β	C-1b ; C-5 ; C-6a C-1b ; C-6a	H-5β
5	42,7	2,96 ; 1H ; d (8,0) 3,32 ; 1H ; m	H-4α H-4β	C-3a ; C-4 ; C-6a C-3a ; C-4 ; C-6a	H-4β
6a	53,3	3,81 ; 1H ; dd (10,8 ; 4,0)	H-7α	C-1a ; C-7	-
7	35,8	2,65 ; 1H ; t (7,2) 2,83 ; 1H ; dd (3,2 ; 1,6)	H-6a	C-1b ; C-6a ; C-8 ; C-11a C-1b ; C-6a ; C-8 ; C-11a	-
7a	-	-	-	-	-
8	110,9	6,79 ; 1H ; s	-	C-7 ; C-9 ; C-10 ; C-11a	H-6a ; H-7β
9	146,6	-	-	-	-
10	144,4	-	-	-	-
11	113,9	7,55 ; 1H ; s	-	C-1a ; C-9 ; C-10	-
11a	124,5	-	-	C-9	-
OCH_3 (C-9)	55,5	3,87 ; 1H ; s	-	-	-
O-CH_2-O (1)	100,4	5,88 ; 1H ; s	O-CH_2-O (2)	C-2	O-CH_2-O (2)
O-CH_2-O (2)	100,4	6,00 ; 1H ; s	O-CH_2-O (1)	C-1	O-CH_2-O (1)

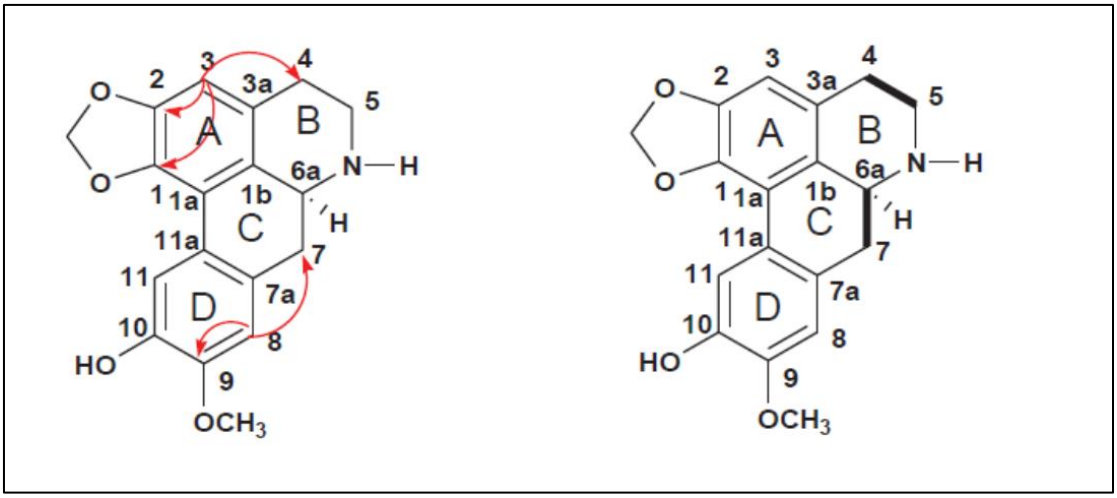

Figure 2 HMBC (⤾) and COSY (—) correlations of (+)-listeferine (MC.4).

4. Discussion

The study of the methanol extract of *Monodora tenuifolia* leaves has formally identified two isoquinoline-type alkaloids. These are (-)-N-methylarmepavine, a benzylisoquinoline, and (+)-listeferine, an oxoaporphine. To our knowledge, these results constitute the first evidence of these two alkaloids in the leaves of *M. tenuifolia*. They are chemotaxonomic markers of the genus, as they have already been isolated from the leaves of *Monodora crispata* and *Monodora brevipes* [2]. The isolation of these two compounds represents a significant advance in understanding the leaf metabolome of this species, which has been little studied [9]. While previous work has mainly focused on seed or root extracts [12], our study highlights the chemical richness of the leaves, which are often overlooked in phytochemical studies.

Structural elucidation based on ^{1}H and ^{13}C NMR was reinforced by two-dimensional COSY, HMBC, and NOESY experiments. COSY correlations established the spin systems of aromatic and aliphatic protons, while long-range connectivities observed in HMBC confirmed the bonds between methyl or methylene protons and aromatic quaternary carbons. The NOESY experiment provided crucial spatial information, particularly for confirming the relative configuration of substituents on the isoquinoline ring.

(-)-N-methylarmepavine, belonging to the benzylisoquinoline class, is known for its antioxidant and antispasmodic activities [13]. Previous studies have shown that this compound also has interesting neuroprotective properties, particularly through the inhibition of acetylcholinesterase [14]. As for (+)-listeferine, a representative molecule of oxoaporphines, it often has antiproliferative and antifungal activities [15, 16]. Its presence in the leaves of *M. tenuifolia* suggests a probable role in the plant's chemical defense against biotic stress.

The combination of these two types of alkaloids in the same plant matrix is particularly interesting from a chemosystematic point of view. It could reflect a diversified adaptive strategy in the *Monodora* genus, but also offer a lead for the development of naturally occurring bioactive compounds for therapeutic use.

5. Conclusion

This study enabled the isolation and characterization of two isoquinoline alkaloids, (-)-N-methylarmepavine and (+)-listeferine, from the leaves of *Monodora tenuifolia* Benth. using ^{1}H NMR, ^{13}C NMR, COSY, HMBC, and NOESY spectroscopic techniques. Rigorous structural analysis of the spectra enabled unambiguous identification of the compounds. The known pharmacological properties of these alkaloids, particularly their antioxidant, antimicrobial, and neuroactive effects, make this species of particular interest for its potential in pharmacognosy. These results contribute to expanding the chemical knowledge of the *Monodora* genus and pave the way for in-depth bioactive studies aimed at evaluating the therapeutic potential of these compounds.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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