wjpmr, 2025, 11(8), 111-123

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH
www.wjpmr.com

SJIF Impact Factor: 6.842

Research Article
ISSN (O): 2455-3301
ISSN (P): 3051-2557

ISOLATION AND STRUCTURAL ELUCIDATION OF THREE NEW COMPOUNDS FROM THE AERIAL PARTS OF *CROTON GÉAYI* LÉANDRI (EUPHORBIACEAE), ENDEMIC TO MADAGASCAR. BIOLOGICAL STUDIES

Ratiankavana Benjamin Larios Princis[1,2*], Ralaivaon-Dratsitonta Jumael Edith Fabrice[1,3], Tiandreny Hazara Jipaty[1], Andrianarijaona Mamy[1,2], Fienena Raymond François[1,2], Fiatoa Barthelemy[1,3], Fatiany Pierre Ruphin[1,2]

[1]Ecole Doctorale Géosciences, Physique, Chimie De L'environnement Et Système De Haute-Pathogènes (GPCEHP), Université De Toliara, Madagascar.
[2]Domaine De Sciences Et Technologies, Université De Toliara, Madagascar.
[3]Centre Universitaire Régional Androy (CURA), Université De Toliara, Madagascar.

***Corresponding Author: Ratiankavana Benjamin Larios Princis**
Ecole Doctorale Géosciences, Physique, Chimie De L'environnement Et Système De Haute-Pathogènes (GPCEHP), Université De Toliara, Madagascar.

Article Received on 25/05/2025 **Article Revised on 15/06/2025** **Article Accepted on 05/07/2025**

ABSTRACT
The genus Croton is involved in several medicinal applications. The species *Croton geayi Léandri*, endemic to southern Madagascar, exhibits antimicrobial and vasorelaxant activities. Chemical studies conducted on the aerial parts of this plant led to the isolation of three novel compounds: two, designated LCB-01 and LCB-02, belonging to the iridoid family, which inhibit bacterial activity; and a third, designated LCB-03, belonging to the indole alkaloid family, which acts as a vasorelaxant. The structures of these three molecules were elucidated using mass spectrometry and 1D and 2D NMR spectroscopy.

KEYWORDS: C. Geayi Léandri; iridoid; antimicrobial; vasorelaxant; biological analysis; chemical study.

1. INTRODUCTION

The southern part of Madagascar is known for its arid conditions and water scarcity. Some plant species have adapted to this harsh climate and have even become valuable in local traditional medicine due to their verified therapeutic properties.

The genus *Croton* is widely recognized in traditional medicine. Several species are used in the treatment of various ailments such as inflammation, asthma[1,2], gastrointestinal infections[3], diabetes, and lipid disorders.[4] In Madagascar, *Croton* species play an important role in traditional healing practices, notably due to their distinctive aromas.[5]

The species *Croton géayi* Léandri is endemic to Madagascar and is found in the southern region of the island. It is known by the vernacular names *kelimanitse* (Antandroy), *pisopisovavy* (Mahafaly), and *fandroadolo* (Sakalava). Ethnobotanical surveys have revealed that the local population uses the leaves for respiratory issues by inhaling the vapor. Traditional healers also report the use of stem bark decoctions to treat hypertension.

To our knowledge, no in-depth phytochemical study has yet been conducted on *Croton géayi* Léandri. However, some biological studies have highlighted the antibacterial activities of the aerial parts and the essential oil extracted from the leaves.[5]

The aerial parts (leafy stems) of *Croton géayi* Léandri were collected on July 8, 2022, in the Menabe region of Madagascar. Following phytochemical screening to identify the main chemical families present in the plant, an extraction was carried out, followed by a protocol that led to the isolation of three pure compounds. Structural elucidation of these compounds revealed three novel molecules.

2. MATERIALS AND METHODS

2.1. Plant material

The aerial parts of *Croton géayi* Léandri were collected on July 8, 2022, in Fenoarivo at GPS coordinates 20°08'N et 44°53'E, Ampasy village (*fokontany*), Ankiliabo rural commune, Manja district, Menabe region, Madagascar.

Figure 1: *Croton géayi* Léandri.

2.2. Phytochemical screening

The first step in the study of a plant consists in identifying the main classes of secondary metabolites it has developed over the course of its evolution. This information is crucial for chemical analyses, as it guides the selection of appropriate extraction methods to avoid the degradation of molecules and the formation of artifacts. It also helps focus on differences in chromatographic analyses used during the isolation process of bioactive compounds.

Subsequently, comparing the results of phytochemical screenings with ethnobotanical and chemotaxonomic data allows the formulation of a hypothesis regarding the basic structure of the active principle.

The classical method of Fong and *al*.[19] was used for phytochemical screening.

2.3. Hydroalcoholic extraction

The aerial part was dried in a dry, sun-protected environment, then ground. Three kilograms of the ground material were macerated twice in an ethanol-water mixture (10/90) at room temperature. The hydroalcoholic solutions were evaporated using a rotary evaporator.

2.4. Preliminary separation

The column chromatography method was used.
Column: a glass column 70 cm in height and 7 cm in diameter.
Stationary phase: silica gel (0.040 mm; 0.063 mm).
Elution: a solvent system with increasing polarity (cyclohexane, hexane/dichloromethane 4/6, ethyl acetate, butanol).
The fraction eluted with hexane/dichloromethane shows the best biological activity.

2.5. Isolation

6 grams of the hexane/dichloromethane extract were separated on a silica gel column, eluted with a dichloromethane/MeOH (8/2) mixture in isocratic mode, followed by fractionation on a Sephadex gel column, eluted with a dichloromethane/MeOH (50/50) mixture, and then further fractionation on a silica gel column eluted with a cyclohexane/EtOAc mixture using a gradient mode. This process resulted in the isolation of three pure compounds, labeled LCB-01, LCB-02, and LCB-03.

2.6. Structural determination

The isolated compounds were analyzed by mass spectrometer, one-dimensional NMR (1H and 13C) and two-dimensional NMR (COSY, HSQC, HMBC).

2.7. Antimicrobial test[8,18]

The bacterial susceptibility test of the extract from the aerial part of Croton géayi Léandri was evaluated using the disk diffusion method on a gel medium. This technique allows for the determination of the bactericidal capacity and the minimum inhibitory concentration (MIC) of the bacteria. It is employed to assess the antimicrobial activities of the plant through antibacterial assays. In this method, the determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) was performed via the microdilution method using 96-well microplates.

2.8. Antihypertensive activity test

Experiments were conducted on isolated rat aortas to elucidate the pharmacological effects of various extracts, fractions, and plant-derived compounds, as well as their underlying mechanisms of action. To facilitate these vascular-level investigations, thoracic aortas from Wistar rats served as the experimental model. The pharmacodynamic mechanisms underlying the activities of these compounds were elucidated through a series of assays assessing their inhibitory effects on contractile responses elicited by specific antagonists and agonists, thereby providing insight into their modes of action at the molecular and receptor levels.[6]

3. RESULTS

3.1. Phytochemical screening

The phytochemical screening indicates a strong presence of the iridoid and steroid families, a notable presence of coumarins, triterpenes, and polyphenols; a low presence of alkaloids; and the absence of polysaccharides, flavonoids, tannins, and anthraquinones.

Table 1: phytochemical screening.

Chemical families	Test	Results
Alkaloids	Mayer, Wagner and Dragendorff	±
Flavonoids	MeOH, Mg, concentrated HCl	-
Coumarins	NaOH, UV lamp λ 254nm and 365nm	++
Anthraquinones	$CHCl_3$ and NH_4OH	-
Leucoanthocyanins	Hot HCl	-
Tannins	H_2O, NaCl, gelatin, $FeCl_3$	-
Saponines	H_2O	-
Polyphenols	Gelatin	++
Triterpenoids	H_2SO_4, CH_2Cl_2, $C_4H_6O_3$, concentrated HCl	++
Stéroids	H_2SO_4, CH_2Cl_2, $C_4H_6O_3$, concentrated HCl	+++
Polysaccharides	Ethanol	-
Iridoids	HCl 12N	+++

3.2. Extraction

The two cold macerations yielded a total of 29.568%.

Table 2: extraction yeld.

	Duration of maceration (h)	Volume (l)	Specific weight (g)	Yeld (%)
	$T_1 = 4$	5	512.71	17.090
	$T_2 = 6$	5	374.35	12.478
Total	$T_t = 10$	10	887.06	29.568

3.3. Isolation

The application of the adsorption chromatography separation technique on the two active fractions obtained from the extract of the hexane and dichloromethane mixture (4/6) allowed the isolation of three pure products.

Table 3: isolated pure products characteristics.

Pure products	Weight (mg)	Y (%) compared to E-Hex/DCM	Color	Solubility
LCB-01	10.0819	0.16803	Crystallisez white	DMSO
LCB-02	8.4827	0.14138	Crystallisez	Methanol
LCB-03	9.0536	0.15089	Colorless	Chloroform

3.4. Antimicrobial activities

The results of the antibacterial screening tests of the various extracts from the aerial part (leafy stem) of Croton géayi Léandri (EUPHORBIACEAE) showed that E. Hex/DCM (4/6) and E.B possess antibacterial activity. Among these two active extracts, the hexane-dichloromethane (4/6) mixture extract demonstrated the better activity against all bacteria, with inhibition zones ranging from 8 to 34 mm at a concentration of 10 µg per disk.

Table 4: antimicrobial test.

Microbial strains	Inhibition zone diameter (mm)							
	Concentration at 5µg/ml				Concentration at 10µg/ml			
	E. CycloHex	E. Hex/DCM	E. ACoET	E.B	E. CycloHex	E. Hex/DCM	E. ACoET	E.B
B. cereus	4.50	**22.35**	3.20	15.42	7.50	**33.65**	6.52	22.5
B. subtilis	4.17	7.04	1.30	5.50	2.58	8.22	4.01	6.50
S. aureus	3.30	**17.06**	4.21	14.21	7.20	**28.15**	7.28	17.01
E. colis	3.20	**14.50**	3.08	11.12	6.52	**23.50**	3.77	16.20
E. cloacae	1.43	7.98	1.90	4.10	3.12	8.18	2.95	7.11
P. aeruginosa	5.13	**16.30**	4.20	12.40	7.80	**28.13**	8.01	18.30
S. Typhii	1.02	6.23	2.31	5.00	3.45	8.60	5.23	6.80
C. albicane	2.53	**18.24**	4.08	11.79	6.13	**26.41**	6.51	12.05

3.5. Vasorelaxant activity of LCB-03 product

The test was performed on the isolated rat aorta, precontracted with phenylephrine at 10^{-6} M. The results with the compound LCB-03 are recorded in the table.

Table 5: Percentage relaxation induced by pure LCB-03 and its EC_{50}.

Pure isolated compound	EC_{50} for relaxation	Percentage (%) of relaxation
LCB-03 (µg/ml)	0.013 ± 0.0007	100

3.6. Structural determination of LCB-01

INTERPRETATION OF THE 1D NMR SPECTRUM OF THE LBC-01 PRODUCT PROTON

The 1D NMR spectral band of the proton in LBC-01 spans from 1 to 6 ppm. The overall shape of this band allows us to conclusively determine that the chemical structure of the LBC-01 molecule does not contain a benzene ring. The interpretation of this spectral band highlights the characteristics and assignments of the protons present in the LCB-01 product.

Four signals specific to methyl protons are identified

- The two proton signals appearing as singlets at 1.70 ppm (3H) and 1.82 ppm (3H) are assigned to the protons of two methyl groups. The other two signals, appearing as singlets at 3.30 ppm (3H) and 3.63 ppm (3H), are shifted downfield relative to the previously identified methyls and are assigned to the protons of methoxy groups;
- A poorly resolved signal in the form of a hump and a singlet, appearing at 4.14 ppm and 4.81 ppm respectively, correspond to protons with acidic properties. These are assigned to two hydroxyl group protons. Additionally, signals from protons with characteristics matching those of non-aromatic alkenic protons are observed at 5.21 ppm (triplet, assigned to the allyl group proton), at 5.93 ppm (singlet, assigned to an alkene proton in a cyclic structure), and two doublets at 5.01 ppm and 5.04 ppm, assigned to geminal alkenic protons in different chemical environments;
- A series of signals at 1.73 ppm (multiplet), 2.02 ppm (double doublet), 2.26 ppm (dd), 2.54 ppm (multiplet), 2.63 ppm (d), 3.22 ppm (dd), 3.26 ppm (d), and 3.89 ppm (d) correspond to protons on cyclic alkyl groups.

INTERPRETATION OF THE 1D NMR SPECTRUM OF THE LCB-01 PRODUCT CARBON

The analysis of the 13C 1D NMR spectrum of the compound LCB-01 was performed in broadband coupled mode with DEPT 135°.

Interpreting the 13C NMR spectrum in DEPT mode indicates that this molecule of LCB-01 contains four methyl carbons, three methylene carbons, and eight sp2 carbons. Nineteen (19) carbon signals were identified.

- Four signals correspond to methyl groups and their derivatives, with the two desaturated signals at δC 18.7 and δC 24.7 assigned to methyl carbons attached to the ethylenic carbon, and the other two, more shielded signals at 51.9 ppm and 57.7 ppm, assigned to carbons of methoxy groups;
- A carbon signal at δC 173.1 ppm is assigned to a carbonyl carbon. Additionally, olefinic carbons appear at 110.8 ppm (assigned to a methylene), at 122.7 ppm and 129.0 ppm (assigned to carbons of the sp2 group), and at 144.6 ppm and 145.7 ppm, attributed to quaternary olefinic carbons;
- A series of signals characteristic of cyclic carbons in the basic structure of this molecule include: δC 32.6 (–CH2), δC 34.8 (CH), δC 35.2 (–CH2), δC 41.9 (–CH), δC 62.1 (–CH), δC 65.1 (–CHOH), δC 72.3 (–CHOH), and δC 95.6 (–CH).

COSY SPECTRUM

The COSY spectrum identifies the protons connected by scalar couplings and confirms the assignment of protons present in the LCB-01 molecule. The results of these COSY spectrum interpretations for the LCB-01 molecule have been recorded in the table 6.

HSQC SPECTRUM

On its HSQC spectrum, it allowed the identification of correlations between the protons and the carbons they are attached to. The results of the interpretation of the HSQC spectrum of LCB-01 have been recorded in the table 6.

Table 6: HSQC ($^{13}C - ^{1}H$) and COSY ($^{1}H - ^{1}H$) correlation of LCB-01.

δH (ppm)	δC (ppm)	Multiplicity	COSY	Degree of substitution
1.70	18.7	s	-	Methyl group attached to an ethylenic
1.73	41.9	m	2.26 ; 3.32 ; 3.89	Cyclic CH
1.82	24.7	s	-	Methyl group attached to an ethylenic
2.02	32.6	dd	2.27 et 2.54	Non equivalent linear alkyl CH_2
2.27		dd	2.02 et 2.54	
2.26	62.1	dd	1.73 et 3.26	Cyclic CH
2.54	34.8	m	2.02 ; 2.27; 3.11 et 3.26	CH in a cycle
2.63	35.2	d	5.21	Allylic CH_2
3.22	95.8	dd	1.73 et 2.54	Cyclic CH
3.26	65.1	dd	2.26 et 2.54	Cycloalcohol CHOH

4.81		massing	-	
3.30	57.7	s	-	Méthyl group attached to oxygen, as in an alcoholate OCH_3
3.63	51.9	s	-	Méthyl group attached to oxygen, as in an ester OCH_3
3.89	72.9	d	1.73	Cycloalkene CHOH
4.14		bulging	-	
5.01	110.8	d	5.04	Non equivalent linear alkene CH_2
5.04		d	5.01	
5.21	122.7	s	-	Allylic CH
5.93	127.0	s	-	Cycloalkene CH

HMBC SPECTRUM

The interpretation of the HMBC spectrum of compound LCB-01 reveals correlations between protons and carbons over long distances, providing the sequence and justifying the connectivity of the molecule.

The first sequence of the LCB-01 molecule is observed from the signals of the ethyl protons.

- The proton signal appearing as a doublet at δH 5.21 correlates with the carbons of two methyl groups, appearing at 18.7 ppm and 24.7 ppm, as well as with the two quaternary carbons at δC 132.2 and 144.6. Additionally, this proton exhibits scalar coupling with protons resonating at 1.63 ppm, attached to the carbon at δC 35.2, and is vicinal to this carbon;
- The two methyl protons, resonating at 1.70 ppm (singlet) and 1.82 ppm (singlet), correlate with the carbon at δC 132.2.

All these correlations allow the determination of the first sequence of the LCB-01 molecule.

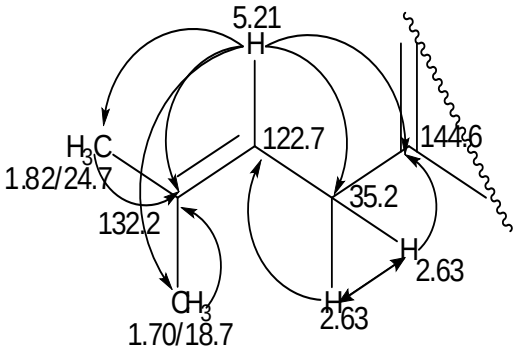

Figure 2: The first sequence of the LCB-01 molecule.

The second sequence of the LCB-01 molecule was determined based on the following correlations:

- The proton signal appearing as a multiplet at 1.73 ppm, attached to the carbon at δC 41.9, correlates with carbons resonating at 62.1 ppm, 72.9 ppm, and 95.8 ppm. Additionally, a weak correlation is observed with the two olefinic carbons at δC 144.6 and δC 145.7;
- The signals of two non-equivalent geminal olefinic protons, appearing as doublets at 5.01 ppm and 5.04 ppm, correlate with carbons at 127.0 ppm (bearing the proton at δH 5.93) and 61.2 ppm (bearing the proton at δH 2.26). These protons do not exhibit scalar coupling with each other, despite being in meta positions relative to each other. Moreover, the proton signal at 5.93 ppm correlates with carbons at 72.3 ppm, 62.1 ppm, and 110.8 ppm;
- The proton signal at δH 2.26, coupled with the two protons at 3.26 ppm attached to the carbon at δC 65.1 and the proton at 1.73 ppm attached to δC 41.9, correlates with the carbon at δC 34.8. However, the proton at δH 2.54 (attached to δC 34.8) is not coupled with the proton at δH 2.26, but shows scalar coupling with the proton at 3.26 ppm, despite being in a meta position relative to the 2.26 ppm proton and vicinal to the 3.26 ppm proton;
- The proton signal at δH 2.54, appearing as a multiplet, correlates with carbons at 32.6 ppm, bearing two non-equivalent geminal protons resonating at 2.02 ppm and 2.27 ppm, as well as with the carbon at 95.8 ppm bearing the proton at δH 3.22. This latter proton at 3.22 ppm shows scalar coupling with the proton at 1.73 ppm attached to δC 41.9, but is not coupled with the proton at 2.54 ppm, even though both are in para positions.

All these correlations, combined with the COSY results, allowed for the elucidation of the second sequence of the molecule in compound LCB-01.

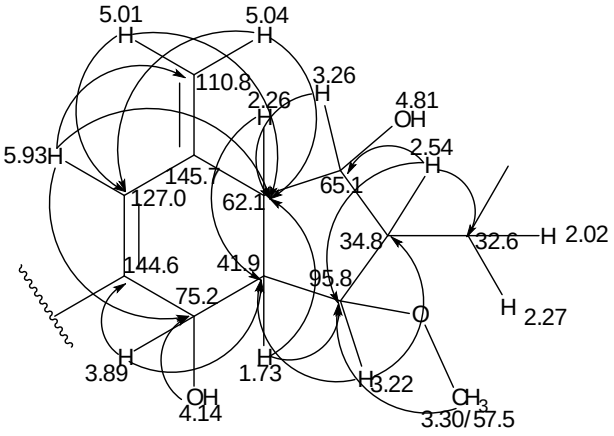

Figure 3: The second sequence of the LCB-01 molecule.

The third sequence of the LCB-01 molecule was elucidated based on the correlation of two geminal

protons appearing as doublets of doublets at 2.02 ppm and 2.27 ppm, respectively, with the carbon of the methylene group at δC 34.8, and with the carbonyl carbon at δC 173.1. The methoxy proton signal at 3.63 ppm correlates with the carbonyl carbon at 173.1 ppm.

All three correlations together allowed for the identification of the third and final sequence of the LCB-01 molecule.

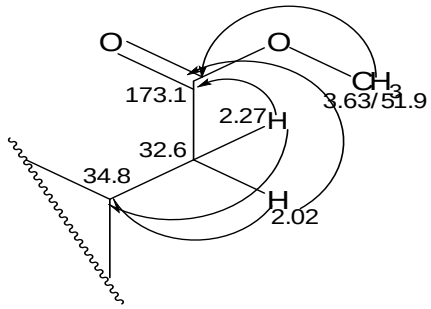

Figure 4: The third sequence of the LCB-01 molecule.

The entire sequence of these three stages results in the final structure of the LCB-01 molecule.

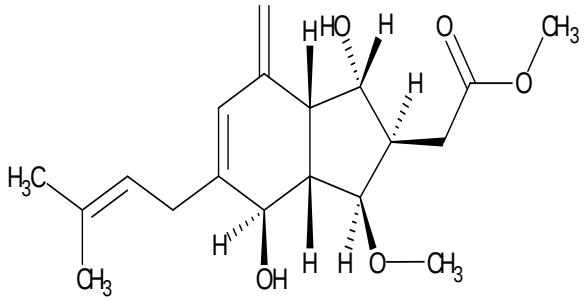

Figure 5: The complete molecular structure of the LCB-01.

3.7. Structural determination of LCB-02

INTERPRETATION OF 1D 1H SPECTRUM

The analysis results obtained by 1D ^1H NMR spectroscopy of the LCB-02 product allowed the identification of twenty-five (25) proton signals, with chemical shifts observed within the spectral range of 0.90 ppm to 12 ppm. The overall pattern of this spectral region provides insights into the various types of proton environments and the structural features of the molecule.

Distinct signals corresponding to specific proton environments characteristic of methyl groups and their derivatives were observed.

- A prominent, long-lasting singlet signal at δH 0.91 ppm integrating for 6 protons, along with two additional singlets at δH 1.70 ppm and 1.82 ppm, each integrating for 3 protons, are attributed to methyl groups. The singlet at δH 1.18 ppm, appearing as a doublet, corresponds to methyl protons attached to a methyne (CH) moiety;
- A series of nine proton signals between δH 1.44 ppm and 3.26 ppm, including multiplets at δH 1.44, 1.51, 1.54, 1.56, 1.79 (dd), 1.90 (m), 2.04 (m), 2.26 (dd), and a doublet of doublets at δH 3.26 ppm, are characteristic of β-ocimenol-type protons, which form the core structural motif of the LCB-02 molecule;
- Additionally, signals indicative of ethyl groups were identified: a doublet of doublets at δH 5.20 ppm corresponding to the alkene proton of the allyl group; two non-equivalent ethylic protons resonating at δH 4.92 ppm and 5.11 ppm, both appearing as doublets and assigned to the protons attached to the same non-equivalent carbon; four signals appearing as doublet of doublets at δH 1.79, 2.00, 2.04, and 2.25 ppm, corresponding to geminal protons in distinct chemical environments neighboring methylene and methyne groups; and a singlet at δH 11.79 ppm assigned to the hydroxyl proton exhibiting acidic properties;
- These proton signals collectively elucidate the structural framework of LCB-02, confirming the presence of methyl, allylic, methylene, and hydroxyl functionalities within the molecule.

INTERPRETATION OF 1D 13C SPECTRUM

The 13C NMR spectrum of compound LCB-02 was obtained through two analytical techniques: the broadband (BB) method and the DEPT 135° experiment. The DEPT 135° spectrum facilitated the identification of five methyl groups, four methylene groups, and ten methine groups. Notably, this method does not detect quaternary carbons. The 13C NMR spectrum acquired via the BB mode revealed the presence of twenty-two carbon signals, as indicated by the vertical trace.

Analysis of these spectra obtained from both the BB and DEPT 135° experiments elucidates the specific characteristics of the twenty-two identified carbons, along with their assignments as follows.

- The carbon resonance at δC 178.9 ppm is assigned to the carbonyl carbon of an acid functional group. The three signals at δC 108.1 ppm (corresponding to a $=CH_2$ group), δC 123.5 ppm (CH), and δC 131.3 ppm (quaternary carbon) are all attributed to carbons within the ethylenic (alkene) moiety;
- The spectrum shows four additional signals at δC 18.6 ppm, δC 21.3 ppm (accounting for two carbons), δC 23.5 ppm, and δC 24.6 ppm, which are assigned to methyl carbons. Furthermore, signals at δC 30.9 ppm and δC 33.9 ppm are attributed to methylene carbons ($-CH_2$ groups);
- A series of signals at δC 28.4 ppm (CH), 39.1 ppm (CH), 40.8 ppm (CH_2), 41.6 ppm (CH), 45.4 ppm (CH), 48.0 ppm (CH), 49.4 ppm (CH), 61.7 ppm (CH), 67.5 ppm (CHOH), and 69.0 ppm (CHOH) are assigned to carbons characteristic of the oplopenol framework, reflecting the compound's specific structural motifs.

This comprehensive spectral analysis provides detailed insights into the carbon skeleton of LCB-02, facilitating further structural elucidation.

COSY SPECTRUM

The COSY spectrum identifies the proton-proton correlation, that is, determining the homonuclear scalar coupling. The results of the COSY spectrum interpretations have been recorded in the table 6.

HSQC SPECTRUM

The interpretation of the HSQC spectrum, combined with that of the DEPT 135° spectrum of LCB-02, defines the degree of substitution of carbons on one hand, and on the other hand, determines the correlations of chemical shifts between protons and the carbon to which they are attached. This allows for the identification of direct correlations between the proton and its corresponding carbon. The interpretation results of these spectra have been recorded in the table 6.

HMBC SPECTRUM

The interpretation of the HMBC spectrum allows for the identification of long-range correlations between protons and carbons, as well as justifying the assignments of the molecular connectivity.

- The proton signal at δH 5.20 (dd) correlates with carbons resonating at 18.6 ppm, 24.6 ppm—attributed to methyl groups—as well as with the carbon bearing the two non-equivalent geminal protons appearing at δC 30.9, and with the two quaternary carbons resonating at 39.1 ppm and 131.3 ppm.

These correlations provide the first sequence of the molecule.

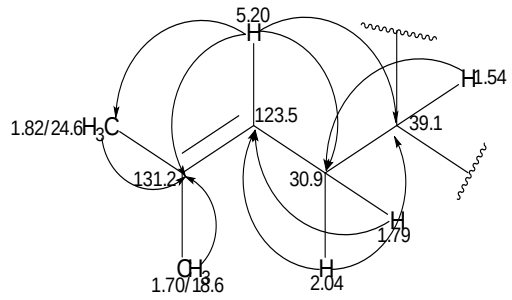

Figure 6: The first sequence of LCB-02 molecule.

- The signals of two non-equivalent geminal protons, appearing at 2.00 ppm (dd) and 2.25 ppm (dd), correlate with the acidic carbonyl at δC 178.9 and with the methylene carbon at δC 48.0. These correlations enabled the determination of the second sequence of the LCB-02 molecule.

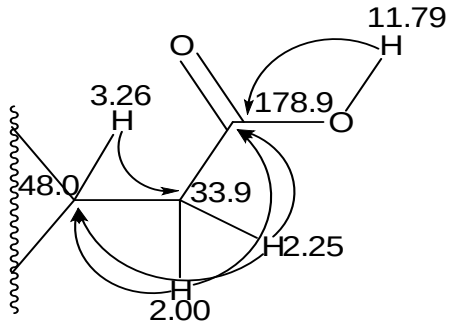

Figure 7: The second sequence of LCB-02 molecule.

The last sequence of the LCB-02 molecule was elucidated based on the correlations of the series of proton signals as follows.

- The two non-equivalent geminal proton peaks at 5.11 ppm (d) and 4.92 ppm (d) correlate with carbons at δC 40.8 and δC 61.7. The carbon at 40.8 ppm bears two non-equivalent geminal protons at 1.79 ppm (dd) and 2.04 ppm (dd), which correlate with carbons at δC 148.0 (quaternary), the carbon at δC 61.7 (bearing the proton at 2.26 ppm), and the carbon at δC 39.1 (bearing the proton at δH 1.54, dd). According to COSY, the proton at δH 1.54 couples with the proton at 1.44 ppm, and this proton at 1.44 ppm shows no scalar coupling with the previously mentioned non-equivalent geminal protons, despite being in meta positions within a saturated ring;
- The proton signal at 1.54 ppm, attached to the carbon at δC 39.1, correlates with carbons at δC 45.4, 30.9, with a weaker correlation to carbons at 28.4 ppm and 41.6 ppm. The proton at 1.90 ppm (multiplet), attached to the carbon at δC 48.0, correlates with carbons at δC 69.0, 33.9, and 49.4, which bears the proton at 1.56 ppm. The proton at 1.56 ppm couples with protons at 1.51 ppm and 1.82 ppm, attached respectively to carbons at δC 41.6 and δC 67.5. Additionally, the proton peak at 1.82 ppm (multiplet) correlates with the methyl carbon at 23.5 ppm, and the proton at 3.26 ppm (dd), attached to the carbon at δC 69.0, correlates with the carbon at δC 61.7.

All these correlations, considering scalar couplings between protons, have enabled the determination of the third sequence of the molecule of compound LCB-02.

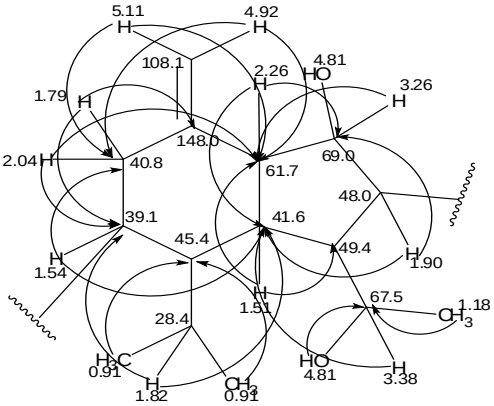

Figure 8: The third sequence of the LCB-02 molecule.

The set of the three sequences results in the complete structure of the pure LCB-02 molecule.

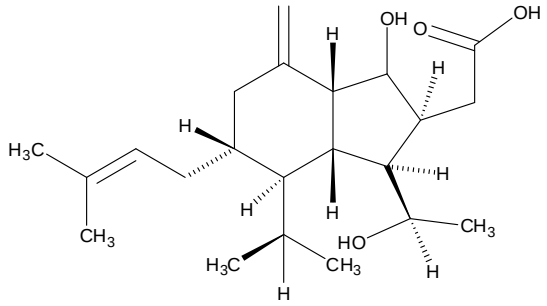

Figure 9: The complete molecular structure of the LCB-02 molecule.

Table 7: Summary of the interpretations of the NMR spectra (1D and 2D) of LCB-02.

Carbon N°	Type	1D NMRexperiments		2D NMR experiments	
		δH	δC	COSY	HMBC
1	CH	1.90	48.0	$H_{1''a}$; $H_{1''b}$; H_2 et H_9	C-3 ; C-2 ; C-1'' et C-9
2	CHOH	3.26	69.0	H_3 et H_1	C-8 ; C-3 et C-1
		4.81		-	C-3
3	CH	2.26	61.7	H_2 et H_8	C-8 ; C-4 ; C-4α et C-3
4	Cq	-	148.0		-
4α	CH_2	4.92	108.1	$H_{3α2}$	C-2 ; C-4 et C-5
		5.11		$H_{3α1}$	C-2 ; C-4 et C-5
5	CH_3	1.70	40.8	H_{5b} et H_6	C-1' ;C-6 ; C-4 et C-4α
		2.24		H_{5a} et H_6	C-6 ; C-1' ; C-4α et C-3
6	CH	1.54	39.1	$H_{1'a}$; $H_{1'b}$; H_{5a} et H_{5b}	C-1' ; C-5 ; C-8 et C-2'
7	CH	1.44	45.4	H_3 ; H_8 ; H_6 et $H_{7α}$	C-6 ; C-8 ; C-3 et C-5
7α	CH	1.82	28.4	H_7 ; $H_{7α1}$ et $H_{7β}$	C-7 ; C-7$α_1$ et C-7β
7$α_1$	CH_3	0.91	21.6	$H_{7α}$	C-7α
7β	CH_3	0.91	21.6	$H_{7α}$	C-7α
8	CH	1.51	41.6	H_9 ; H_3 et H_7	C-3 ; C-7 et C-9
9	CH	1.56	49.4	H_8 ; H_1 ; $H_{9α}$	C-1, C-8 et C-9α
9α	CHOH	3.38	67.5	H_9 et $H_{9β}$	C-9 et C-9β
		4.81		-	C-9α
9β	CH_3	1.18	23.5	$H_{9α}$	C-9α
1'	CH_2	2.04	30.9	$H_{2'}$	C-6 et C-2'
		1.79		$H_{2'}$	C-2' et C-6
2'	CH	5.20	125.5	$H_{1'a}$ et $H_{1'b}$	C-4'; C-3' ; C-6 et C-3'α
3'	Cq	-	131.3	-	-
3'α	CH_3	1.70	18.6	-	C-3'
4'	CH_3	1.82	24.6	-	C-3'
1''	CH_2	2.00	33.9	H_1 et $H_{1''b}$	C-1 ; C-2'
		2.25		H_1 et $H_{1''a}$	C-1 ; C-2''
2''	OCOH	11.79	178.9	-	C-2''

3.8. Structural determination of the LCB-03 molecule
INTERPRETATION OF 1D 1H NMR SPECTRUM
Five characteristic signals are identified.

- The three singlet signals at δH 1.79, δH 1.82, and δH 1.86 are all attributed to methyl group protons;
- The proton signal appearing as a doublet at 1.11 ppm is assigned to the methyl proton adjacent to the CH;
- The deshielded proton signal at δH 3.80 is attributed to the methoxy group proton.

Five other characteristic signals corresponding to the properties of the protons in the benzene ring appear at δH 7.33 (d), δH 6.41 (d), δH 6.97 (d), δH 7.93 (d), and δH 8.03 (s). Additionally, four signals observed at δH 3.38 (d), δH 4.08 (d), δH 5.75 (s), and δH 5.79 (s) are all assigned to ethyl protons.
We also noted the presence of three proton signals at δH 3.21 (d), δH 3.73 (dd), and δH 3.98 (dd), which are attributed to methylene group protons. The last two are geminal non-equivalent protons.

INTERPRETATION OF 1D ^{13}C NMR SPECTRUM

The results of the ^{13}C NMR analysis (both broadband and DEPT 135°) of compound LCB-03 revealed the presence of twenty-two carbon atoms, including a notably deshielded and unshielded carbon signal at δC 160.9, which is attributed to the enolate functionality. The interpretation of these spectra allows for the identification of various characteristic features and assignments of the carbons within this compound.

- The highly characteristic proton signals correspond to the chemical environments of methyl groups and their derivatives ;
- Four signals observed at δC 17.2, 18.6, 20.1, and 24.6 ppm are assigned to methyl carbons, while the most prominent deshielded carbon signal at δC 58.6 ppm is attributed to the methoxy group carbon ;
- Eight signals are indicative of the indole core, appearing at δC 100.9 (CH), 126.7 (CH–N), 112.2 (H), 120.2 (CH), 120.9 (CH), 125.5 (CH), 136.0 (CH), and 136.4 (Cq–N). Additionally, five signals at δC 80.3, 112.5, 123.1, 131.0, and 141.9 ppm are assigned to the carbons of linear alkenes. Finally, three signals corresponding to alkyl carbons are observed at δC 34.0 (CH_2), 60.5 (CH_2), and 39.4 ppm (CH).

COSY SPECTRUM

The COSY spectrum allows for the identification of proton-proton correlations, i.e., to determine homonuclear scalar coupling. The results of the COSY spectrum interpretations have been recorded in the table 8.

HSQC SPECTRUM

The interpretation of the HSQC spectrum, combined with that of the DEPT 135° spectrum of LCB-03, allowed for determining the degree of substitution of carbons on one hand and, on the other hand, for establishing the correlations between proton chemical shifts and the dominant carbon signals. In other words, it enabled the identification of the direct correlation between a proton and its attached carbon. The results of the spectral interpretations are summarized in the table 8.

Table 8: HSQC (^{13}C – ^{1}H) and COSY (^{1}H – ^{1}H) correlations of the LCB-03 molecule.

δH (ppm)	Multiplicity order	δC (ppm)	Carbon caracteristics	COSY
1.11	m	39.4	CH	3.01 ; 3.73 ; 3.98
1.70	s	18.6	CH_3	-
1.82	s	24.6	CH_3	-
1.86	s	17.2	CH_3	-
3.01	d	20.1	CH_3	1.11
3.21	d	34.0	CH_2	5.75
3.80	s	58.6	CH_3	-
3.73	dd	60.5	CH_2	1.11 ; 3.98
3.98	dd			1.11 ; 3.73
3.83	d	80.3	CH_2	4.08
4.08	d			3.83
5.75	d	123.1	CH	1.11
5.79	s	112.5	CH	-
6.41	d	100.9	CH	7.33
6.97	d	120.2	CH	7.93
7.33	d	126.7	CH	6.41
7.93	d	120.6	CH	6.97
8.03	s	111.2	CH	-

HMBC SPECTRUM

The HMBC spectrum reveals correlations between protons and carbons over long distances, typically in α and β positions, which helps to establish the connectivity of the molecule. Indeed, the initial sequence of the LCB-03 molecule is identified from the olefinic proton at δ 5.75, which.

- The proton signal at δH 5.75 (t) correlates with carbons resonating at 18.6 ppm and 24.6 ppm, attributed to methyl groups, as well as with the methylene carbon at δC 34.0 and the quaternary aromatic carbon at δC 136.0.

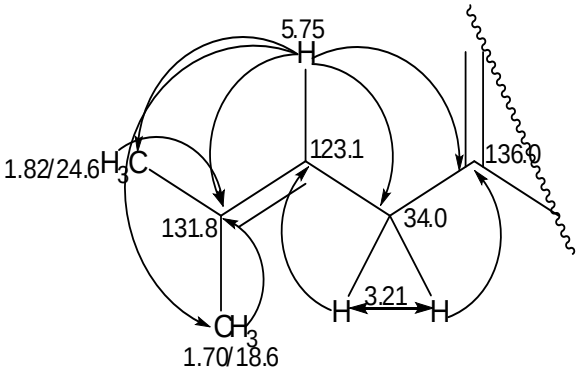

Figure 10: The first sequence of LCB-03 molecule.

The second sequence of the LCB-03 molecule is observed from the correlations involving the aliphatic protons, which are assigned to the protons of the indole core.

- The proton appearing as a singlet at 8.03 ppm, attached to the carbon at δC 111.2, is correlated with three carbons: at 34.0 ppm (CH_2), at 120.2 ppm (CH), and at 125.5 ppm (a quaternary benzene carbon). Additionally, the proton signal as a doublet at δH 7.93 correlates with carbons at 136.0 ppm and 136.4 ppm; both are quaternary carbons of the benzene ring. This proton is correlated with the carbon at δC 100.9 ppm (CH). Since this proton couples with the proton at 6.97 ppm but shows no scalar coupling with the proton at 8.03 ppm, these two protons are in a para position;
- The proton appearing at 6.97 ppm as a doublet, assigned to the aliphatic proton of the benzene ring, is correlated with carbons at 34.0 ppm, 111.2 ppm, and 125.5 ppm. Furthermore, the proton signal as a doublet attached to the carbon at δC 100.9 ppm, which appears at 6.41 ppm, correlates with carbons at δC 120.6 and δC 136.4. According to COSY, this proton couples with the proton at 7.33 ppm attached to the carbon at δC 126.7, and this proton at δH 7.33 correlates with the carbon at 125.5 ppm, with a weak correlation to carbons at δC 60.5 and δC 136.4.

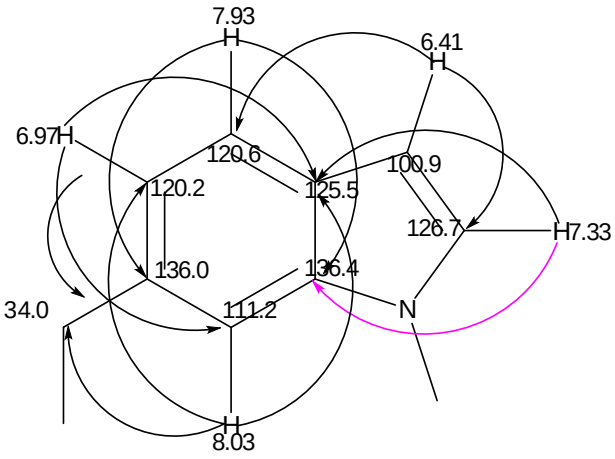

Figure 11: The second sequence of the LCB-03 molecule.

The third sequence of the LCB-03 molecule was elucidated based on the correlation of two non-equivalent geminal protons appearing at 3.73 ppm (dd) and 3.98 ppm (dd). These proton signals correlate with the carbon of the methyne group at δC 39.4, which bears a proton at 3.01 ppm. Subsequently, the proton signal at 3.01 ppm correlates with the methyl carbon at δC 20.1 and also with the quaternary alkene carbon at δC 141.9, which carries a methyl group that appears at 17.1 ppm. Moreover, the alkene protons in different chemical environments, appearing at 3.83 ppm (d) and 4.08 ppm (d), correlate with carbons at δC 112.5 (alkenic CH), δC 141.9 (alkenic quaternary carbon), and δC 160.9 (enolate carbon). The methoxy proton signal at 3.80 ppm correlates with the enolate carbon at δC 160.9.

All these correlations above allowed for the identification of the third and final sequence of the LCB-03 molecule.

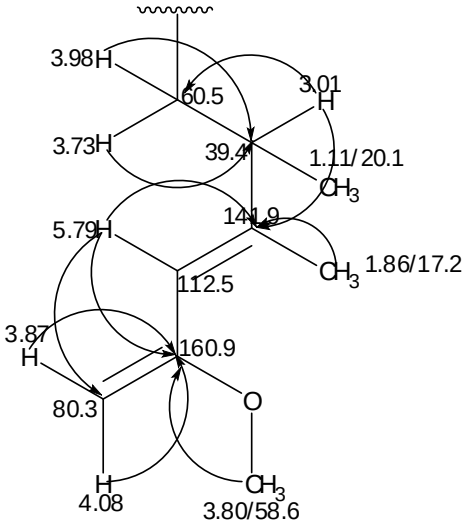

Figure 12: The third sequence of the LCB-03 molecule.

The combination of the three sequences provides the complete structure of the pure product molecule, LCB-03.

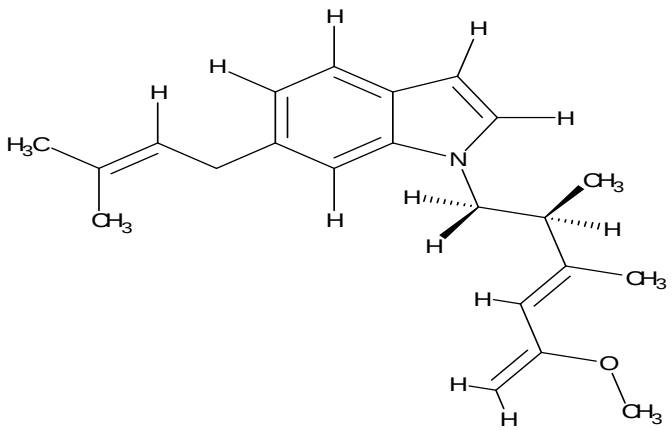

Figure 13: The complete structure of the LCB-03.

Table 9: Summary of the interpretations of the NMR spectra (1D and 2D) of the LCB-03 molecule.

Carbon N°	Type	1D NMR experiments		2D NMR experiments	
		δH	δC	COSY	HMBC
1a	Cq	-	136.4	-	
2	CH	7.33 (d)	126.7	H_1	C-3a et C-4
3	CH	6.41(d)	100.9	H_2	C-3a et C-1a
3a	Cq	-	125.5		
4	CH	7.93 (d)	120.6	H_5	C-1a et C-6
5	CH	6.97 (d)	120.2	H_4	C-3a et C-7
6	Cq	-	136.0		
7	CH	8.03 (s)	111.2	-	C-1'' ; C-5 et C-3a
1'	CH_2	3.73 (dd)	60.5	$H_{1'b}$; $H_{2'}$	C-2'
		3.98 (dd)		$H_{1'a}$;$H_{2'}$	C-2'
2'	CH	1.11 (m)	39.4	$H_{1'a}$; $H_{1'b}$; $H_{2'a}$	C-1', C-3' et C-2'a
2'a	CH_3	3.01 (d)	20.1	$H_{2'}$	C-2'
3'	Cq	-	141.9		
3'a	CH_3	1.86 (s)	17.2	-	C-3'
4'	CH	5.79 (s)	112.5	-	C-2' ; C-3', C-3'a, C-5' et C-6'
5'	Cq	-	160.9		
5'a	CH_2	3.83 (d)	80.3	$H_{5'a2}$	C-4' et C-5'
		4.08 (d)		$H_{5'a1}$	C-4' et C-5'
5'b	O- CH_3	3.80 (s)	58.6	-	C-5'
1"	CH_2	3.21 (d)	34.0	$H_{2''}$	C-2'' et C-6
2"	CH	5.75 (t)	123.1	$H_{1''}$	C-3''a, C-3''b et C-6
3"	Cq	-	131.8		
3"a	CH_3	1.70 (s)	18.6	-	C-3''
3"b	CH_3	1.82 (s)	24.6	-	C-3''

4. DISCUSSIONS

Higher plants have the ability to synthesize, through complex metabolic pathways, numerous compounds that they utilize for various adaptive functions; notably in response to biotic and abiotic stresses they may encounter. Plants therefore contain a wide variety of molecules with different physico-chemical properties, which exhibit diverse biological activities. These activities depend on their systematic, botanical, and especially their geo-ecosystem classifications, including environmental and ecological factors. Moreover, it is now widely recognized that plants serve as inexhaustible sources for human health, making bioactive molecules particularly important. Indeed, industrial sectors (cosmetics, pharmaceuticals, and agro-food) are increasingly turning to the incorporation of these naturally derived molecules, especially medicinal plants.

Ethnobotanical surveys conducted in the southwestern part of Madagascar, particularly in the Menabe region, have revealed that the aerial part of a plant known by the vernacular name "Fandroadolo," a dialect of the Sakalava tribe, scientifically called Croton géayi Léandri (Euphorbiaceae), is used in traditional medicine to treat infections, blood pressure issues, and fevers. Biological screening tests show that this plant exhibits notable antibacterial activity and a very promising vasorelaxant effect. These results support the ethnomedicinal data regarding this plant.

The application of bioguided fractionation techniques led to the isolation of three pure active principles. The two compounds labeled LCB-01 and LCB-02 possess antibacterial activities, while the third, labeled LCB-03, demonstrates a vasorelaxant effect. The chemical structures of these compounds were elucidated using mono- and bi-dimensional NMR spectroscopy and mass spectrometry. The basic structures of LCB-01 and LCB-02 belong to the iridoid family, whereas LCB-03 is classified among indole alkaloids. Stereochemical studies performed on these three molecules revealed that each contains at least one chiral carbon. The molecule LCB-02 has non-racemic isomers identified at carbon number 14, which bears a labile proton attributed to a tertiary alcohol.

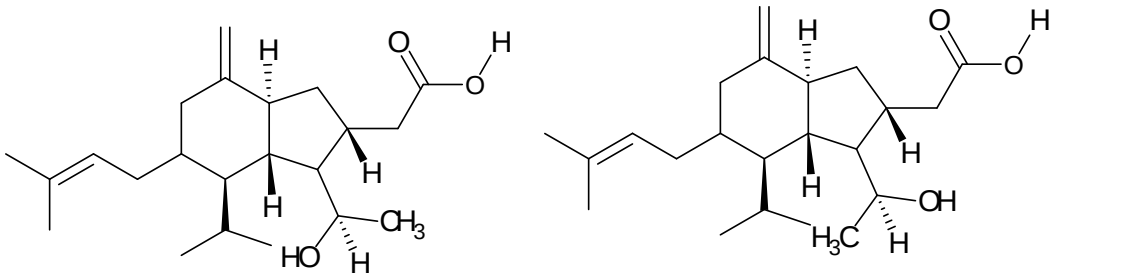

Figure 13: The stereochemistry of the LCB-02 molecule.

The bibliographic studies conducted on these three molecules have shown that they are not described in the literature nor in the context of endemic Croton species from Madagascar. All three molecules are novel.

The results of the biological studies on the product LBC-03 indicate that it possesses vasorelaxant effects, with an EC50 on isolated rat aorta of 0.013 ± 0.0007 µg/mL. Regarding the pharmacological activity mechanisms of LCB-03, the results of tests on its inhibitory effects against contractions induced by agonists and antagonists show that LCB-03 induces a concentration-dependent relaxing effect on precontracted rat aorta with phenylephrine at 10^-6 M, both in the presence and absence of endothelium. This indicates that the relaxing effect of this molecule is endothelium-dependent. Furthermore, tests involving other inhibitors revealed that the activity of LCB-03 does not involve the NO pathway, β2-adrenergic receptors, or prostacyclin. Additionally, tests were performed to assess the inhibitory effect of LCB-03 on calcium-induced contractions of isolated rat aorta in a depolarizing, calcium-free medium. Membrane calcium channel opening was induced by depolarizing vascular smooth muscle cell membranes through increased extracellular K+ concentration (a depolarizing environment). The concentration-effect results, both with and without LCB-03, showed that the compound could inhibit calcium-induced contraction in a concentration-dependent manner. This suggests that the molecule's relaxant activity may result from blocking calcium influx through the vascular smooth muscle cell membrane, as calcium-induced contraction of rat aorta in a depolarizing, calcium-free medium was inhibited by LCB-03 in a concentration-dependent manner.

The other two molecules, LCB-01 and LCB-02, belong to the same chemical family. Both are active and exhibit bactericidal properties. However, their activities differ, with LCB-02 being more active than LCB-01. The differences in activity may be due to the presence of asymmetry at C-14 and the saturation of the bond between C-5 and C-6.

5. CONCLUSION

Ethnobotanical surveys conducted at the collection site of Croton geayi Léandri provided detailed insights into the plant's utilized parts, preparation methods, and modes of administration for therapeutic purposes. These findings facilitated the development of standardized protocols for the extraction and isolation of the plant's bioactive constituents. Consequently, three novel molecules were isolated and structurally characterized using spectroscopic techniques; notably, two exhibited antimicrobial activity, while the third demonstrated vasorelaxant properties. The preliminary structure-activity relationship analyses discussed herein suggest meaningful correlations that warrant further in-depth pharmacological and mechanistic investigations.

6. REFERENCES

1. Secco R., Notas sobre as lianas do gênero *Croton L.* (Euphorbiaceae), Bol. Mus. Para. Emilio Goeldi, serie Botanica, 1992; 8(2): 265-281
2. Salatino and al., Traditional uses, Chemistry and Pharmacology of Croton species (Euphorbiaceae), Journal of the Brazilian Chemical Society, 2007; 18(1): 11-33
3. Campos G.E.R. and al., Muscular adaptations in response to three different resistance-training regimens: specificity of repetition maximum training zones, Eur J Appl Physiol, 2002; 88(1-2): 50-60.
4. Grassi B. and al., Muscle oxygenation and pulmonary gas exchange kinetics during cycling exercise on-transitions in humans, J Appl Physiol, 2003; 95(1): 149-158.
5. Fatiany P.R. and al., GC-FID and GC/MS analyses and Antimicrobial activity of *Croton greveanus, C. borarium* and *C. geayi* (Euphorbiaceae) essential oils from Madagascar, Journal of Pharmacognosy and Phytochemistry, 2016; 5(4): 188-197.
6. Fiatoa B., Ratiankavana B.L.P. and al., Spasmolitic effects of Kauranoid and ester of linear alkaloid from the aerial part of *Croton Borarium* (Euphorbiaceae) endemic in South part of Madagascar, International Journal of Progressive Sciences and Technologies, 2024; 46(2): 351-374.
7. Ratiankavana B.L.P. and *al.*, Isolation and structural elucidation of two new compounds from the leaves of *Cnestis polyphylla* (Connaraceae) of Madagascar, World Journal of Advance Pharmaceutical Sciences, 2025; 2(2).
8. Rios J.L. et al., Medicinal plants antimicrobial activity, J Ethnopharmacol., 2005; 100(1-2): 80.
9. Declume C. et Assamoi A. Etude comparative de l'activité d'élimination des polyphénolsévaluée par

des méthodes différentes. Journal Chimique d'Agriculture Alimentaire, Pub Med, 1984; 47: 425–431.
10. Fatiany P. R., 2015, Recherche de molécules bioactives sur les extraits et les mélanges complexes volatiles issus de quelques espèces de plantes médicinales et aromatiques du Sud-ouest de Madagascar. Mémoire Habilitation à diriger des Recherches. Université de Toliara, Madagascar.
11. Fatiany P.R., Robijaona B, Claude M, Koto-te-Nyiwa-Ngbolua JP, 2014, Quelques composés chimiques d'intérêt biopharmaceutiques isolés de trois plantes médicinales de Madagascar. Forum de Recherche à Benin.
12. Fatiany PR, Abdallah M, René R, Ratsimamanga SU, 2013, Activité antihypertenseur de *Cymbopogon giganteus Madagascariensis* (Poacée). Communication orale. Forum de la Recherche à Mahajanga, BioMad III, p 33.
13. Chassagne F. and *al*., A systematic review of plants with antibacterial activities: a taxonomic and phylogenetic perspective, Frontiers in Pharmacology, 2021; 11: 1-29.
14. Parmard C.B., Ramiarantsoa H.R., 2003, Madagascar : Les enjeux environnementaux, Afrique « Vulnérabilité et défis », Collection question de géographie. Nante Edition du temps, 354-376.
15. Schwartz G.E., 2001, Flore générique des arbres de Madagascar. Royal Botanical Garden, Kew and Missouri Botanical Garden.
16. Pernet R., Meyer G., 1951, Pharmacopée de Madagascar. Instit. De Recherche Scient- Tananarive Tsimbazaza Madagascar.
17. Houngue U.M., 2021, Caractérisation de nouvelles molécules vasorelaxantes : étude chimico-pharmacologique de carissa edulis, une espèce médicinale de la pharmacopée béninoise. Médecine humaine et pathologie. Université de Strasbourg; Université d'Abomey-Calavi (Bénin), Thèse de doctorat.
18. VEYSSIERE A.J., 2019, La résistance aux antibiotiques des bactéries les plus communément rencontrées dans les infections communautaires. Etat des lieux en 2019, thèse de doctorat en pharmacie, Université de Bordeaux.
19. Fong H., Tin Wa M., Farnsworth N.R., 1977, *Phytochemical screening plants*, Documents of Pharmacognosy and Pharmacology, Illinois, Chicago.
20. Vercauteren J., 2018, Pharmacognosie, spéciale-Drogues à alcaloïdes et Phytochimie des plantes médicinales.