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ISOLATION AND STRUCTURAL ELUCIDATION OF TWO NEW COMPOUNDS FROM THE LEAVES OF *CNESTIS POLYPHYLLA* (CONNARACEAE) OF MADAGASCAR

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ABSTRACT

Two new molecular compounds have been discovered in the leaves of Cnestis polyphylla (Connaraceae), collected in Madagascar. This plant has verified acute toxicity, which justifies its empirical use. Biological tests, phytochemical screening, guided extraction, and column isolation were performed on the plant. The determination of the structures of the two molecules was carried out using 1D and 2D NMR.

KEYWORDS: *Cnestis polyphylla*, Isolation, toxicity, structures.

1. INTRODUCTION

Medicinal plants play an important role in human life. They serve as source of medecines, either through direct use or through the extraction of active constituents within pharmaceutical environments, or as nutritional condiments. According to the World Health Organization, more than 80% of the world's population uses plant-based remedies for primary healthcare.[1,2]

The *Cnestis* genus is extensively utilized across Africa due to its diverse therapeutic and pharmacological properties.[3,4,5,6,12] *Cnestis polyphylla* is commonly found in Madagascar; however, its application is restricted, primarily because of its significant toxicity.[7] *Cnestis polyphylla* leaves collected from the Manombo area, Farafangana district, in December 2022 were evaluated using the Brine Shrimp lethality assay, followed by phytochemical screening to identify the chemical families present. Fractionation of one extract allowed the isolation of two pure compounds, which were characterized through spectroscopic techniques.

1. MATERIALS AND METHODS

1.1. Plant material

The leaves of *Cnestis polyphylla* (Connaraceae) were collected near the Manombo Special Reserve, in the Farafangana district, Atsimo Atsinanana region, along the RN12A at PK252, in southeastern Madagascar. The plant was identified by Rolland RANAIVOJAONA, a botanist at the Tsimbazaza Botanical and Zoological Park. A voucher specimen has been deposited there under the number TAN2529.

1.2. Phytochemical screening

Phytochemical screening of *Cnestis polyphylla* leaves was performed following the standard procedure described by Fong et *al.*[8]

1.3. Bioassay Screening of Crude Extracts

This test involves exposing *Artemia salina* larvae (Crustacea) to the extract to be tested, dissolved at a known concentration in seawater. To do this, 10 larvae, hatched in seawater for 48 hours, are transferred into a tube. The extract, at a known concentration, is introduced into the tube (10 mL of extract). Three tubes are prepared for each concentration. After 24 hours, the surviving larvae are counted to evaluate the mortality percentage for each extract concentration.[9] The lethal dose LD_{50} is calculated using Finney’s Probit Analysis program. The extract is considered toxic if its LD_{50} is below 500 µg/mL.

1.4. Extraction

The leaves of *Cnestis polyphylla* were dried and ground. A preliminary maceration in ethanol was performed on 100 g of plant powder to remove pigments and fats from the material. A hot extraction using a solvent gradient was then carried out on this powder (hexane, dichloromethane, diethyl ether, ethyl acetate, acetone, absolute ethanol, and methanol). The extraction was bio-guided: a *Brine Shrimp* toxicity test was conducted on each extract. Extracts exhibiting the highest toxicity were selected for further in-depth study.

1.5. Fractionation and isolation

Low-pressure liquid chromatography performed on the ethyl acetate extract allowed the fractionation and isolation of two pure compounds, labeled LGF-01 and LGF-02, with respective masses of 4 mg and 5 mg. The quantities obtained were insufficient for toxicity testing but adequate for structural determination.

1.6. Structural determination

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

2. RESULTS

2.1. Toxicity testing of the Plant

Cnestis polyphylla exhibits acute biological toxicity, with an LD_{50} of 115 µg/mL. This could explain its traditional use as a poison to kill stray dogs in the village. Each extract was also subjected to the *Brine Shrimp* toxicity test.

Table 1: Toxicity test of the extracts.

Extract	MeOH	EtOH	Acetone	AcOEt	CH_2Cl_2
LD_{50} (µg/ml)	83	842	90	196	500

2.2. Phytochemical screening

The results of the phytochemical screening indicate a high presence of quinones, polysaccharides, hydrolyzable tannins (gallotannins or ellagitannins), and unsaturated sterols. Steroids and flavonols are also present. Alkaloids and saponins are notably absent.

Table 2: Phytochemical screening of *Cnestis polyphylla* leaf powders.

Chemical families	Test	Observation
Alkaloids	Wagner	-
	Mayer	-
	Dragendorf	-
Flavonoids and leucoanthocyanins	Wilstater	Purple +
	Modified Wilstater	Purple +
	Bate Smith I	-
	Bate Smith II	-
Tannins and polyphenols	Gelatin	-
	Salted Gelatin	++

	$FeCl_3$ in MeOH	Bluish black +++
Quinones	NH_4OH ½	+++
Steroids and terpenoids	Libermann Burchard	Blue-green ++
	Salkowski	Red +++
	Badjet Kedde	-
	Keller Killiani	-
	Saturated antimony	Yellow ++
Saponins	Foam	-
Polysaccharides	3 volumes of alcohol	+++

2.3. Structural determination of LGF-01

The 1D ^1H NMR spectrum of compound LGF-01 shows proton chemical shifts in the spectral range between 2 ppm and 12 ppm. Four specific proton characteristics present in this compound were identified:

- A very broad singlet appears at δH 2.15; the integration of this peak corresponds to three protons, indicating the presence of a methyl group attached to a benzene ring;
- Two multiplet signals appear at 2.50 ppm and 2.75 ppm, respectively, clearly attributed to methylene group protons ($–CH_2–$) in different chemical environments;
- Three doublet-of-doublets appear at δH 6.39, δH 4.97, and δH 4.05. Additionally, two doublets observed at 5.66 ppm and 6.51 ppm are methine protons, all attributed to protons of the pyran ring system;

Next, the spectrum revealed the presence of aromatic ring protons with distinct characteristics:

- Five singlet signals at δH 6.58, 6.78, 6.92, 6.98, and 7.05, along with six doublet peaks at 6.61 ppm, 6.77 ppm, 6.80 ppm, 6.82 ppm, 6.99 ppm, and 7.14 ppm, are all attributed to olefinic protons of the benzene ring. In addition, two doublet signals at δH 5.04 ppm and δH 5.89 ppm are assigned to protons of linear alkenes. These assignments are supported by the general chemical shift table, which indicates that linear alkene protons typically appear between 4.68 ppm and 6.45 ppm.

Finally, the proton signals observed at 9.63 ppm, 9.81 ppm, 10.02 ppm, 10.68 ppm, and 11.85 ppm can be attributed to phenolic or acidic protons. According to the general chemical shift table, mobile protons with acidic character typically appear in the range of 9 to 13 ppm. Since the chemical shift values of these protons fall within this range, they are therefore assigned to phenol and acid protons.

The interpretation of the ^13C NMR spectrum of compound LGF-01, recorded in BB (broadband decoupling) mode, enabled the identification of thirty-three (33) carbon atoms.

In addition, the DEPT spectrum (Distortionless Enhancement by Polarization Transfer) revealed the presence of one methylene carbon, eighteen methine carbons, and one methyl carbon. However, the ^13C NMR spectrum recorded in DEPT mode does not allow the identification of quaternary carbons. Based on the interpretation of the one-dimensional ^13C NMR spectra in both BB (broadband decoupling) and DEPT modes, compound LGF-01 contains thirteen quaternary carbon atoms. The analysis of these spectra allowed the identification of carbon signals along with their corresponding assignments:

- Five carbon signals were identified at δC 149.1, 149.3, 155.0, 158.4, and 165.0, all attributed to enolic carbons with acidic properties, such as those found in phenolic groups. The presence of these carbons is supported by the phenolic proton signals observed in the ^1H 1D NMR spectrum;
- Two highly characteristic carbon signals were observed at δC 75.7 and δC 80.5, attributed to carbons bonded to heteroatoms, likely from the halogen group. However, the phytochemical screening results of the plant matrix indicate the absence of alkaloids, suggesting instead that these signals correspond to C–O carbons of the chromane ring.
- In addition, nine carbon signals were identified at δC 113.8, 121.8, 122.9, 132.5, 132.7, 139.1, 140.0, 147.2, and 149.4, assigned to ethylenic quaternary carbons of benzene rings.
- Five characteristic carbon signals were also observed at 110.6 ppm, 113.1 ppm, 114.0 ppm, 114.5 ppm, and 115.6 ppm, attributed to CH carbons of benzene rings. In addition, six peaks at δC 113.7, 116.7, 118.3, 120.6, 129.2, and 130.6 were assigned to carbons of para-disubstituted benzene rings.

Five distinct carbon signals were identified:

- The signals at δC 104.4 and δC 165.0 are attributed to the alkenic carbons of a hydroxyvinyl group, while the signal at 15.5 ppm is assigned to the methyl group (CH_3) carbon;
- The carbon peaks at δC 121.7 and δC 126.5 are attributed to the ethylenic carbon signals of the chromane ring.

The COSY spectrum allowed the identification of scalar couplings (^1H–^1H correlations) between protons, revealing connectivity within the molecule. Notably:

- The proton at δH 6.82 (doublet) is coupled with the proton at δH 6.77 (doublet).

- The proton at δH 4.97 (doublet of doublets) is coupled with two nonequivalent geminal protons appearing as multiplets at δH 2.50 and 2.75. These geminal protons are also coupled with the proton at δH 4.05 (doublet of doublets).
- The proton at δH 6.61 (doublet) shows coupling with the proton at δH 7.16 (doublet).
- The proton at δH 5.04 (doublet) correlates with the proton at δH 6.24 (doublet).
- The proton at δH 5.66 (doublet of doublets) is coupled with the proton at δH 6.39 (doublet of doublets), which itself correlates with the proton at δH 6.51 (doublet of doublets).
- Finally, the proton at δH 6.80 (doublet) shows a correlation with the proton at δH 6.99 (doublet).

These correlations outline the proton network within the compound, supporting the proposed structural framework. Further HSQC and HMBC analyses complement these findings by establishing heteronuclear correlations that assist in assigning carbon-proton connectivities and elucidating quaternary carbon positions.

Table 3: HSQC (^13C–^1H) and COSY (^1H–^1H) Correlations of Compound LGF-01.

δ (^{1}H) ppm and Proton Type	Multiplicity	δ (^{13}C) ppm	COSY Correlations
2.15 (methyl proton)	S (singlet)	15.1	—
2.50 (nonequivalent methylene proton)	M (multiplet)	49.2	2.75; 4.05; 4.97
2.75 (nonequivalent methylene proton)	M (multiplet)	—	2.50; 4.05; 4.97
4.05 (methine proton of chromane ring)	Dd (dd)	34.0	2.50; 2.75
4.97 (OCH proton of chromane ring)	Dd (dd)	75.7	2.50; 2.75
5.04 (vinyl group proton)	D (doublet)	104.2	6.24
5.66 (OCH proton of chromane ring)	D (doublet)	80.5	6.39
6.24 (vinyl group proton)	D (doublet)	165.0	5.04
10.02 (hydroxy proton of vinyl group)	Broad peak	—	—
6.39 (alkenic proton of chromane ring)	Dd (dd)	126.5	5.66; 6.51
6.51 (alkenic proton of chromane ring)	D (doublet)	121.7	6.39
6.58 (benzenic proton)	S (singlet)	110.6	—
6.61 (benzenic proton)	D (doublet)	120.1	7.16
6.77 (benzenic proton)	D (doublet)	113.1	6.82
6.78 (benzenic proton)	S (singlet)	115.6	—
6.80 (benzenic proton)	D (doublet)	116.7	6.99
6.82 (benzenic proton)	D (doublet)	114.0	6.77
6.92 (benzenic proton)	S (singlet)	114.5	—
6.98 (benzenic proton)	S (singlet)	118.3	—
6.99 (benzenic proton)	D (doublet)	130.6	6.80
7.05 (benzenic proton)	S (singlet)	113.7	—
7.16 (benzenic proton)	D (doublet)	129.2	6.61
9.63 (phenolic OH)	S (singlet)	149.1	—
9.81 (phenolic OH)	S (singlet)	149.3	—
10.68 (phenolic OH)	Broad peak	155.0	—
11.85 (phenolic OH)	S (singlet)	158.4	—

The interpretation of the HMBC spectrum of compound LGF-01 reveals long-range correlations between protons and carbon atoms, typically across two or three bonds (α, β, and occasionally γ positions). These correlations are crucial for establishing molecular connectivity and support the proposed structure through observed fragmentation patterns consistent with mass spectrometry data.

The first proposed fragmentation pathway (Fig. 1) was identified based on correlations involving three alkenic protons from the aromatic (benzene) core:

- The proton at δH 6.77 (doublet), attached to the carbon at δC 113.1, shows long-range correlations with a quaternary carbon at δC 132.7 and with δC 149.1, which bears a hydroxyl group. According to the COSY spectrum, this proton is scalar-coupled to the proton at δH 6.82, assigned to δC 114.0. This indicates a vicinal relationship between the two protons;
- The proton at δH 6.82 (doublet), in turn, shows HMBC correlations with a quaternary carbon at δC 147.8 and with δC 114.5, which carries a proton resonating at δH 6.92 (singlet). Interestingly, this δH 6.92 proton does not correlate with δC 113.1 (bearing δH 6.77), but does correlate with δC 34.0 (bearing δH 4.05, dd), δC 114.0, and δC 147.8. This suggests that the δH 6.92 proton is in the para position relative to the carbon at δC 113.1;
- The two non-equivalent protons at δH 2.50 and 2.75, bound to a methylene carbon at δC 49.2, exhibit HMBC correlations with carbons at δC 75.7 (bearing δH 4.97, dd) and δC 34.0 (bearing δH 4.05, dd). These protons are also scalar-coupled in the

COSY spectrum to the latter two protons, confirming vicinal relationships and consistent chemical environments;

- The δH 4.05 proton, attached to δC 34.0, shows HMBC correlations with δC 49.7, 132.5, and 132.7, supporting its position adjacent to key structural motifs of the chromane core;
- Finally, the proton at δH 4.97, attached to δC 75.7, correlates with carbons at δC 49.7 and 139.1, further supporting the proposed structure and fragment connectivity.

Based on all the above correlations, the first fragmentation pathway of compound LGF-01 can be proposed.

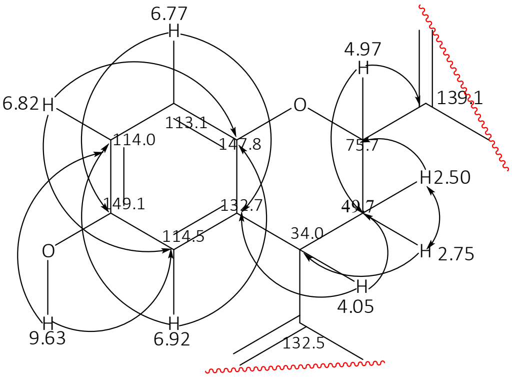

Figure 1: The first fragmentation pathway of the LGF-01 molecule.

The second fragmentation pathway of compound LGF-01 was established based on HMBC correlations between alkenic protons of the benzene core and their attached substituents:

- The proton signal at δH 7.16 (doublet), bound to the carbon at δC 129.2, shows correlations with carbons at δC 104.4 (a vinyl carbon bearing a proton at δH 5.04, doublet), δC 139.1 (a quaternary carbon), and δC 158.4 (bearing a hydroxyl proton at δH 11.85);
- According to the COSY spectrum, the proton at δH 7.16 shows a scalar coupling with the proton at δH 6.61, which is attached to δC 120.6. These two vicinal protons are located on adjacent carbon atoms. Additionally, the δH 6.61 proton (doublet) exhibits long-range correlations with δC 75.7 (a pyran ring carbon), δC 113.8 (a quaternary carbon), and δC 113.9, which is bonded to a proton resonating at δH 7.05. This δH 7.05 proton does not correlate with δC 129.2 (bearing δH 7.16), but rather shows correlations with δC 75.7, δC 113.8, and δC 120.6, suggesting that it is in a para position relative to δC 129.2;
- Two vinyl group protons further support this fragmentation: the proton at δH 5.04 (doublet), attached to δC 104.4, shows correlations with δC 113.8, δC 129.2, δC 158.4, and δC 165.0. Meanwhile, the proton at δH 6.24 (doublet), bound to δC 165.0, correlates with carbons at δC 104.4 and δC 113.8.

Together, these observed correlations confirm the second fragmentation pathway of the LGF-01 molecule.

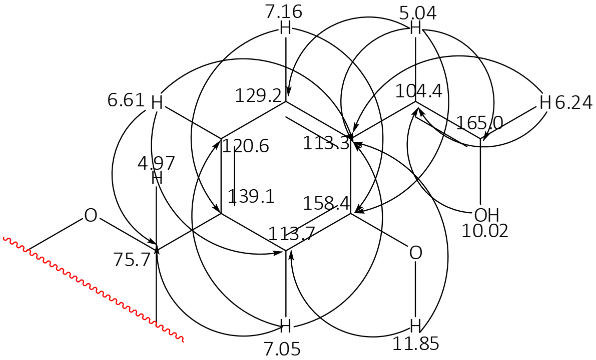

Figure 2: The second fragmentation pathway of LGF-01 molecule.

By combining the two fragmentation pathways described above, the initial structural sequence of the LGF-01 molecule can be established.

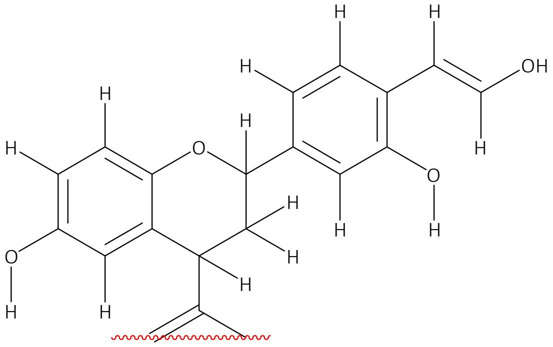

Figure 3: The first structural sequence of the LGF-01 molecule.

The third fragmentation of the LGF-01 molecule was identified based on HMBC correlations involving the proton at δH 6.78 (singlet), attached to the carbon at δC 115.6. This proton shows long-range correlations with the following carbons:

- δC 34.0 (a methine CH carbon);
- δC 110.6 (bearing a proton at δH 6.58);
- δC 149.4 (a quaternary alkenic carbon of the benzene ring); and
- a weak correlation with δC 132.5.

In addition, the proton at δH 6.58 (attached to δC 110.6) correlates with carbons at δC 115.6, 121.7, and 149.4, but not with δC 132.5. This suggests that the proton at δH 6.58 is located para to the carbon at δC 132.5.

Further support for this fragmentation is provided by correlations involving two alkenic protons of the pyran ring:

- The proton at δH 6.51 (doublet) shows correlations with δC 149.4 (quaternary), δC 126.5 (alkenic carbon of the pyran ring, bearing a proton at δH 6.39), δC 110.6 (alkenic carbon of the benzene ring bearing δH 6.58), and δC 80.5 (OCH);
- The proton at δH 6.39 (doublet of doublets), attached to δC 126.5, correlates with δC 80.5, δC 121.7, δC 128.1, and shows a weak correlation with δC 140.0;
- The proton at δH 5.66 (doublet), bound to δC 80.5, exhibits correlations with δC 126.5 and δC 140.0.

Together, these HMBC correlations support and define the **third fragmentation pathway** of the LGF-01 molecule.

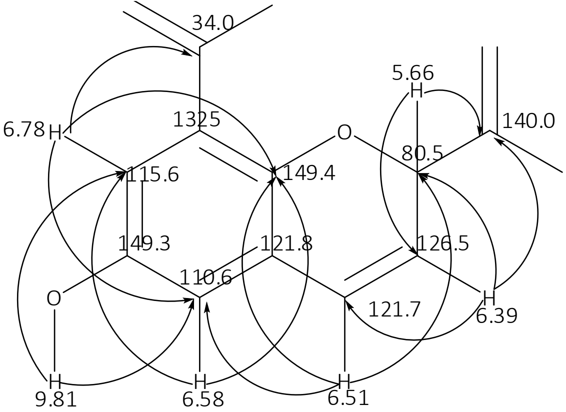

Figure 4: The third fragmentation pathway of the LGF-01 molecule.

The final fragmentation of the LGF-01 molecule was identified based on HMBC and COSY correlations involving three aromatic protons observed at δH 6.80 (doublet), δH 6.92 (singlet), and δH 6.99 (doublet):

- The proton at δH 6.80 (doublet), attached to the carbon at δC 116.7, shows correlations with δC 80.5 (OCH), δC 118.3 (bearing the proton at δH 6.92), and δC 122.9 (a quaternary alkenic carbon of the benzene ring);
- According to the COSY spectrum, this proton is scalar-coupled to the proton at δH 6.99, indicating a vicinal relationship. Additionally, the proton at δH 6.99 (doublet) correlates with δC 15.5 (a methyl group carbon), δC 140.0 (a quaternary alkenic carbon of the benzene ring), and δC 155.0, which bears a hydroxyl proton resonating at δH 10.68;
- The proton at δH 6.92 (singlet), attached to δC 118.3, shows HMBC correlations with δC 80.5 (OCH), δC 116.7 (CH of the aromatic ring), and δC 122.9 (quaternary alkenic carbon of the benzene ring). Notably, it does not correlate with δC 130.6, despite being para-positioned relative to it;
- The methyl group proton at δH 2.15 (long singlet) shows a correlation with the quaternary carbon at δC

122.9. Additionally, the hydroxyl proton at δH 10.68 exhibits weak correlations with carbons at δC 118.3 and δC 122.9.

Taken together, these correlations define the **fourth and final fragmentation pathway** of the LGF-01 molecule.

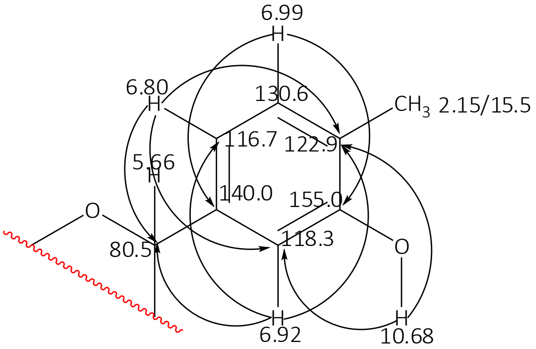

Figure 5: The fourth fragmentation pathway of the LGF-01 molecule.

By combining the two most recent fragments described above, the second structural sequence of the LGF-01 molecule is established.

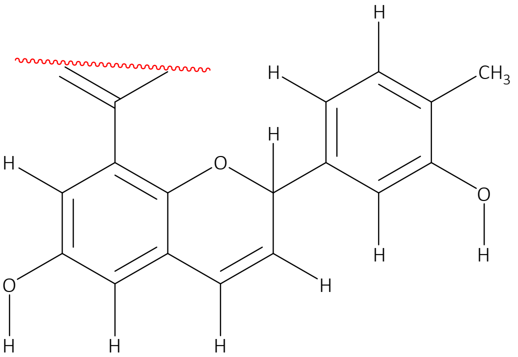

Figure 6: The second structural sequence of the LGF-01 molecule.

The combination of the two sequences leads to the complete molecular structure of the compound LGF-01.

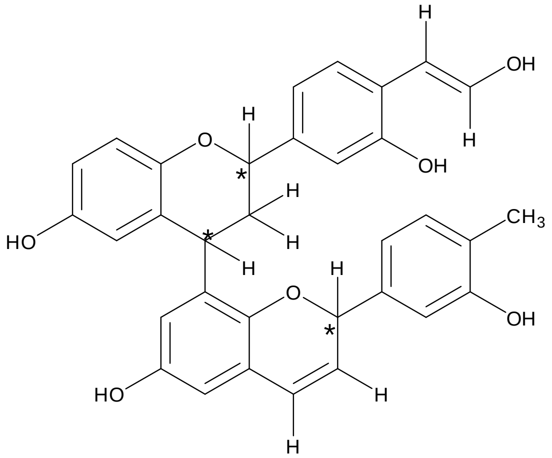

Figure 7: The complete molecular structure of the LGF-01.

Table 4: Summary of the interpretations of the 1D and 2D NMR spectra of the LGF-01.

Carbone No.	Type	1D NMR experiments (^{1}H and ^{13}C)		2D NMR experiments	
		δH	δC	COSY	HMBC
2	OCH (chromane ring)	4.97 (dd)	75.7	[H_{3a}], [H_{3b}]	C-1' and C-3
3	CH$_2$ (non equivalent geminal protons)	2.50 (m)[H_{3a}]	49.2	H_2,H_{3b},H_4	C-2 and C-4
		2.75 (m) [H_{3b}]		H_2, H_{3a}, H_4	C-2 and C-4
4	CH	4.05(dd)	34.0	[H_{3a}], [H_{3b}]	C-3, C-5 and C-9''
5	Cq (benzene)	-	132.7		

6	CH (benzene)	6.92 (s)	114.5	-	C-4, C-8 and C-10
7	C-OH (phenol)	9.63	149.1	-	C-6 and C-8
8	CH (benzene)	6.82 (d)	114.0	H_9	C-6 and C-10
9	CH (benzene)	6.77 (d)	113.1	H_8	C-5 and C-7
10	Cq (benzene)	-	1478		
1'	Cq (benzene)	-	139.1		
2'	CH (benzene)	7.05 (s)	113.9	-	C-2, C-4’ and C-6’
3'	C-OH (phenol)	11.85	158.4	-	C-2’ and C-4’
4'	Cq (benzene)	-	113.8		
4’α	CH (Vinyl)	5.04(d)	104.4	$H_{4'\beta}$	C-3’, C-4’, C-5’ and C-4'β
4'β	HCOH (Vinyl)	6.24 (d)	165.0	$H_{4'\alpha}$	C-4’ and C-4’α
		10.02 (s)		-	C-4’α
5'	CH (benzene)	7.16 (d)	129.3	$H_{6'}$	C-1’ and C-3’
6'	CH (benzene)	6.61 (d)	120.6	$H_{5'}$	C-2, C-2’ and C-4’
2"	OCH (chromane ring)	5.66 (d)	80.5	$H_{3''}$	C-3’’ and C-1’’’
3"	CH	6.39 (dd)	126.5	$H_{2''}$ et $H_{4''}$	C-2’’, C-4’’ and C-1’’’
4"	CH	6.51 (d)	121.7	$H_{3''}$	C-2’’, C-6’’ and C-10’’
5"	Cq (benzene)	-	128.1		
6"	CH (benzene)	6.58 (s)	110.6	-	C-4”, C-8” and C-10”
7"	C-OH (phenol)	9.81	149.3	-	C-6” and C-8”
8"	CH (benzene)	6.78 (s)	115.6	-	C-4, C-6” and C-10”
9"	Cq (benzene)	-	132.5		
10"	Cq (benzene)	-	149.4		
1"'	Cq (benzene)	-	140.0		
2"'	CH (benzene)	6.92 (s)	118.3	-	C-2”, C-4”’ and C-6”’
3"'	C-OH (phenol)	10.68	155.0	-	C-2”’ and C-4”’
4"'	Cq (benzene)	-	122.9		
4"'α	CH_3 (methyl)	2.15 (s)	15.5	-	C-4”’
5"'	CH (benzene)	6.99 (d)	130.9	$H_{6'''}$	C-1”’, C-3”’ and C-4"'α
6"'	CH (benzene)	6.80(d)	116.7	$H_{5'''}$	C-2”, C-2”’ and C-4”’

2.4. Structural determination of LGF-02

The chemical shifts of the protons in compound LGF-02 appear in the spectral range between 1 ppm and 11 ppm. The overall pattern of this range provides insight into the nature of the protons present in this compound:

- Signals between δH 1 and 4 ppm are attributed to alkyl group protons;
- Protons in the range of δH 4 to 6 ppm are attributed to alkene protons and/or linear hydroxyl protons;
- Signals between δH 6 and 8 ppm are assigned to aromatic (benzene ring) protons;
- Peaks from δH 8 to 11 ppm are attributed to labile phenolic protons with acidic properties.

Interpretation of the 1D ^1H NMR spectrum of compound LGF-02 allowed for the identification of specific characteristics for each proton and their respective assignments:

- Three doublet signals at δH 1.53 ppm, 1.68 ppm, and 2.05 ppm are all attributed to methyl protons (CH_3-). Additionally, two singlet signals at δH 4.47 ppm and 4.69 ppm are assigned to methylene protons (CH_2-);
- The signals at δH 3.81 ppm (quartet) and 5.64 ppm (singlet) correspond to methine protons bonded to carbonyl ketone groups;

Four signals were identified as linear alkene (allylic) protons:

- Two doublets at δH 6.25 ppm and 6.42 ppm;
- Two multiplets at δH 4.63 ppm and 5.19 ppm.

Thirteen signals were clearly assigned to aromatic protons:

- Five singlets at δH 7.12, 7.21, 7.26, 7.31, and 7.42 ppm;
- Eight doublets at δH 6.70, 7.03, 7.28, 7.32, 7.35, 7.39, 7.41, and 7.71 ppm.

Two peaks at δH 9.48 ppm and 9.64 ppm correspond to phenolic protons, while the signals at δH 5.56 ppm and 5.61 ppm are assigned to linear alcohol protons.

The 1D ^{13}C NMR spectrum is similar to the proton spectrum, but differs in terms of natural abundance. The ^{13}C NMR analysis of compound LGF-02 was carried out using two acquisition modes:

- BB mode (Broad Band), and
- DEPT mode (Distortionless Enhancement by Polarization Transfer).

The ^{13}C NMR (BB mode) analysis of compound LGF-02 revealed that the molecule contains forty-one (41) carbon atoms, without taking into account any possible symmetry in the structure.

The DEPT ^{13}C NMR spectrum showed that LGF-02 contains:

- Nineteen methine carbons (=CH-);
- Two methylene carbons ($-CH_2-$); and
- Three methyl carbons ($-CH_3$).

Interpretation of the spectra allowed for the identification and assignment of each carbon signal in the compound: Five specific carbon signals were identified:

- Two signals at δC 206.0 and 207.1 ppm, attributed to carbonyl carbons;
- Three signals at 12.5 ppm, 15.4 ppm, and 17.4 ppm, corresponding to methyl group carbons.

Additionally

- Signals at δC 65.5 and 68.7 ppm were assigned to methylene carbons ($-CH_2-$);
- Signals at δC 47.9 and 74.6 ppm were assigned to methine carbons bonded to carbonyl functions;
- The carbon signals at δC 99.8 ppm, 106.5 ppm, 142.8 ppm, and 147.6 ppm were attributed to alkene carbons in allyl groups;
- A series of carbon signals were identified at:103.5 ppm, 107.5 ppm, 108.4 ppm, 110.4 ppm, 111.6 ppm, 115.7 ppm, 116.2 ppm, 120.1 ppm, 120.8 ppm, 121.8 ppm, 122.1 ppm, 123.4 ppm, 125.3 ppm, 128.1 ppm, 129.1 ppm, 129.8 ppm, 130.9 ppm, 132.8 ppm, 133.7 ppm, 136.4 ppm, 141.5 ppm, 142.5 ppm, 145.7 ppm, 151.3 ppm, 154.4 ppm, and 154.6 ppm — all of which are attributed to aromatic (benzene ring) alkene carbons.

The COSY spectrum of LGF-02 identified proton–proton correlations and multiplet patterns. The results of this interpretation are compiled in Table 3.

The HSQC spectrum provided:

- Clear correlations between ^1H and ^{13}C chemical shifts;
- Determination of the degree of substitution of carbons, as detailed in table 5

Table 5: HSQC (^{13}C – ^1H) and COSY (^1H – ^1H) Correlation of the LGF-02 Molecule.

δH (ppm)	δC (ppm)	Multiplicity	COSY Correlations	Substitution Type
1.53	17.4	d	3.81	$-CH_3$
1.68	15.4	d	5.19	$-CH_3$
2.05	12.5	d	4.63	$-CH_3$
3.81	47.9	q	1.53	CH (linear)
4.47	65.5	s	-	$-CH_2OH$
5.56	-	-	-	-
4.63	99.8	m	2.05 and 6.25	=CH– (allylic alkene)
4.69	68.8	s	-	$-CH_2OH$
5.61	-	broad	-	-
5.19	106.5	m	1.68 and 6.42	=CH– (allylic alkene)
5.64	74.6	s	-	CH (linear)
6.25	147.6	d	4.63	=CH– (allylic alkene)
6.42	142.8	d	5.19	=CH– (allylic alkene)
6.70	116.3	d	7.29	Aromatic CH (AA type)
7.03	115.7	d	7.71	Aromatic CH
7.12	111.6	s	-	Aromatic CH
7.21	108.4	s	-	Aromatic CH
7.26	107.5	s	-	Aromatic CH
7.29	120.8	d	6.70	Aromatic CH
7.31	110.3	s	-	Aromatic CH
7.32	129.8	d	7.39	Aromatic CH (disubstituted ring)
7.35	121.8	d	7.41	Aromatic CH
7.39	122.1	d	7.32	Aromatic CH
7.41	128.8	d	7.35	Aromatic CH
7.42	103.5	s	-	Aromatic CH
7.71	127.9	d	7.03	Aromatic CH
9.48	136.2	s	-	Phenol OH
9.64	144.7	s	-	Phenol OH

Interpretation of the HMBC spectrum of compound LGF-02 reveals long-range correlations between protons and carbons (α, β, and even up to γ positions relative to the proton-bearing carbon). This analysis supports the connectivity of the molecule and provides information on its fragmentation.

The HMBC spectrum interpretation establishes the correlations between protons and carbons and gives insight into the molecular sequence.

The identification begins with the correlations involving aromatic protons in an adjacent AA-type ring system:

- The proton signal appearing as a doublet at δH 7.29 correlates with carbons at 136.2 ppm (bearing a hydroxyl proton at δH 9.48), a quaternary carbon at 123.4 ppm, and a carbon at 111.6 ppm bearing a proton at δH 7.12. According to the COSY spectrum, this proton couples with the proton at δH 6.70, indicating they are vicinal. Furthermore, the proton at δH 6.70 correlates with carbons at 120.1 ppm and 130.9 ppm, but not with the carbon at δC 111.6, suggesting a para position relative to it;
- The proton signal at 7.12 ppm correlates with carbons at 145.7 ppm and 123.4 ppm (quaternary aromatic carbons), and with the carbon at 120.8 ppm bearing the proton at δH 7.29;
- The singlet proton signal at 7.42 ppm, carried by the carbon at δC 103.5, correlates with carbons at 141.2 ppm, 130.9 ppm, and 120.1 ppm—all quaternary carbons of the aromatic ring.

All these correlations allowed the identification of the first fragmentation of the LGF-02 molecule.

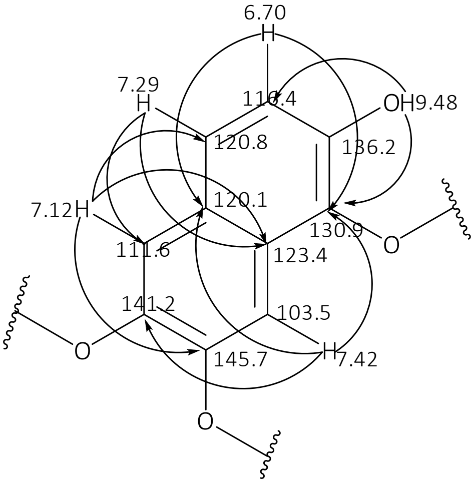

Figure 8: The first fragmentation pathway of the LGF-02 molecule

The second fragmentation of the LGF-02 molecule is identified from the correlations of the alkenic protons of the allyl group. The proton signal appearing as a doublet at 6.42 ppm, carried by the carbon at δ 142.8, correlates with carbons at 141.2 ppm, 106.5 ppm, and 15.4 ppm. According to the COSY spectrum, this proton is coupled with the proton at 5.19 ppm, which is attached to the carbon at δ 106.5, indicating they are vicinal. Additionally, the multiplet proton signal at δH 5.19 correlates with the carbon at 15.4 ppm (a methyl group) and with the carbon bearing the proton at 6.42 ppm. Moreover, it is coupled with the protons of the methyl group.

All the above information made it possible to determine the second fragmentation of the LGF-02 molecule.

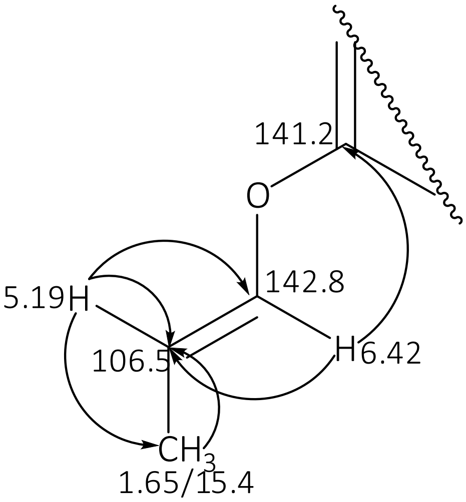

Figure 9: The second fragmentation pathway of the LGF-02 molecule.

The third fragmentation of the LGF-02 molecule is observed through the correlation of alkenic protons from a disubstituted benzene ring, appearing at δH 7.35 and δH 7.41, carried by carbons resonating at 121.8 ppm and 128.8 ppm, which exhibit axial symmetry relative to the substitution bonds. The doublet proton signal at 7.35 ppm correlates with carbons at 133.7 ppm and 121.8 ppm. The doublet signal at 7.41 ppm also shows correlations with carbons at δC 154.4 and δC 47.9.

The quartet proton signal at 3.81 ppm, carried by the asymmetric carbon at δC 47.9 of a methine group, correlates with carbons at 17.4 ppm, 154.4 ppm, 207.1 ppm, and shows a weak correlation with the carbon at 68.8 ppm, attributed to a –CH_2OH group.

All the correlations mentioned above, combined with symmetry considerations, enabled the elucidation of the third fragmentation of the LGF-02 molecule.

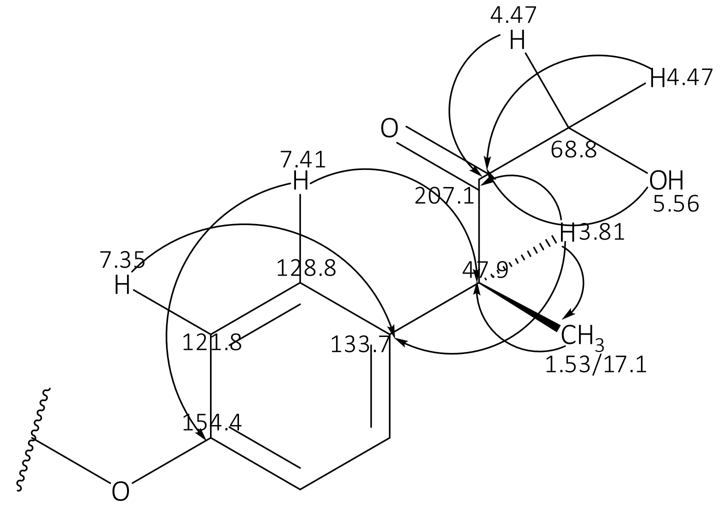

Figure 10: The third fragmentation pathway of the LGF-02 molecule.

The combination of the three fragments identified above has made it possible to elucidate the first sequence of the LGF-02 molecule.

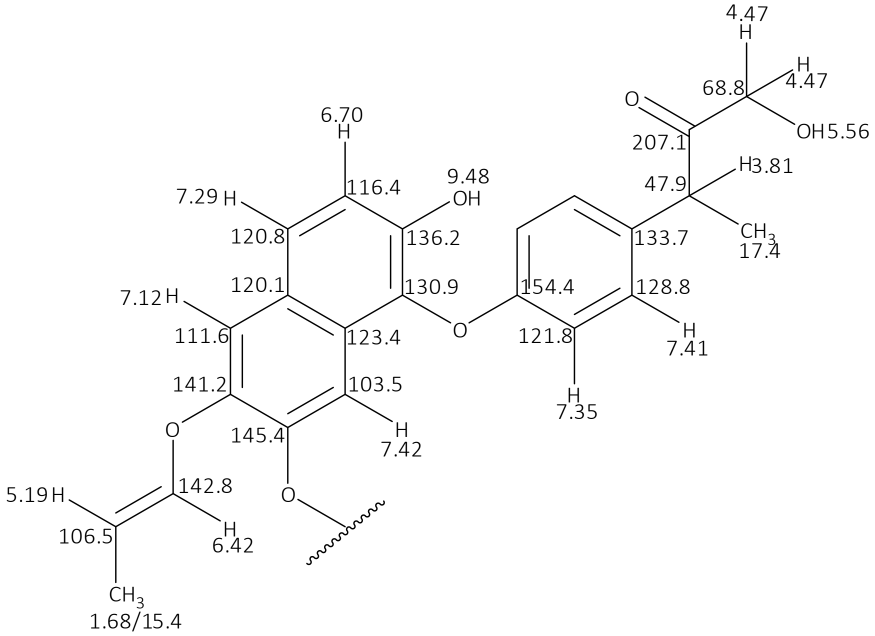

Figure 11: The first structural sequence of the LGF-02 molecule.

The fourth fragmentation of the LGF-02 molecule was identified based on the correlation of alkenic protons from a benzene ring of the AB type:

- The proton signal appearing as a doublet at δH 7.03, carried by the carbon at 115.7 ppm, correlates with two quaternary carbons at 107.5 ppm and 125.3 ppm. According to the COSY spectrum, this proton shows scalar coupling with the proton at δH 7.71. The proton at 7.71 ppm correlates with carbons at 151.3 ppm, 132.8 ppm, and 110.3 ppm, which carries the proton at δH 7.31 (singlet);
- The proton signal at δH 7.31 correlates with carbons at 144.7 ppm (bearing a hydroxyl group), 132.8 ppm

(a quaternary benzenic alkenic carbon), and 127.9 ppm (a benzenic CH).

Correlations are also observed for two proton signals appearing as singlets at δH 7.21 and 7.26:

- The signal at δH 7.21 correlates with carbons at 125.3 ppm, 107.5 ppm, and 142.5 ppm;
- The signal at δH 7.26 correlates with carbons at 115.7 ppm, 125.3 ppm, and 108.4 ppm;

By combining all the above correlation data, the fourth fragmentation of the LGF-02 molecule is established.

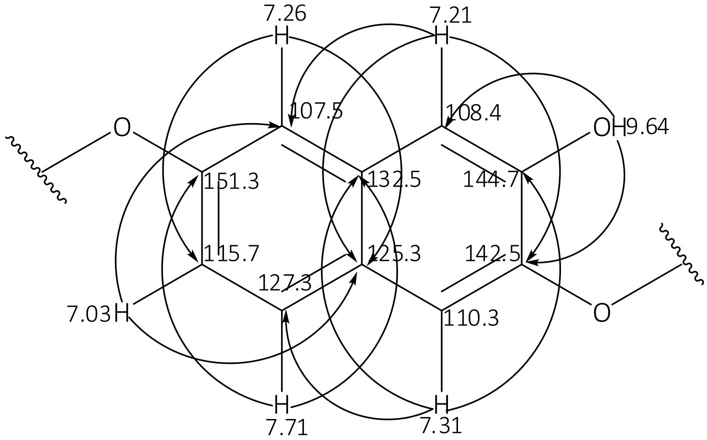

Figure 12: The fourth fragmentation pathway of the LGF-02 molecule.

The last fragmentation of the LGF-02 molecule is observed from the correlation of alkenic protons of the allylic group appearing at 6.25 ppm (d), 4.63 ppm (m), and 2.05 ppm (d):

- The proton signal appearing as a multiplet at 4.63 ppm shows correlations with carbons at 12.3 ppm (bearing the proton at 2.05 ppm) and 147.6 ppm (bearing the proton at 6.25 ppm). According to COSY, the proton at 4.63 ppm exhibits scalar coupling with the two protons appearing at 6.25 ppm and 2.05 ppm, these two protons being vicinal to the proton at 4.63 ppm;
- The proton signal appearing as a singlet at 5.64 ppm, carried by the asymmetric carbon at δC 74.6, correlates with carbons including: the carbonyl carbon at 206.0 ppm, the quaternary alkenic carbon of the benzene ring at 128.1 ppm, and the carbon of the hydroxymethylene group (-CH2OH-) at δC 65.5;
- Correlations are also observed from alkenic protons of the di-substituted benzene ring appearing at δH 7.32 and 7.39 ppm, carried by carbons at 129.8 ppm and 122.1 ppm, showing axial symmetry with respect to the substitution bonds. The proton signal as a doublet at 7.32 ppm correlates with carbons at 154.6 ppm and 74.6 ppm; additionally, the proton signal at 7.39 ppm (also a doublet) correlates with the carbon at 128.1 ppm and shows a weak correlation with the quaternary carbon at 154.6 ppm.

The combination of these correlations, completed by considerations of symmetry, allowed elucidation of the last fragmentation of the LGF-02 molecule.

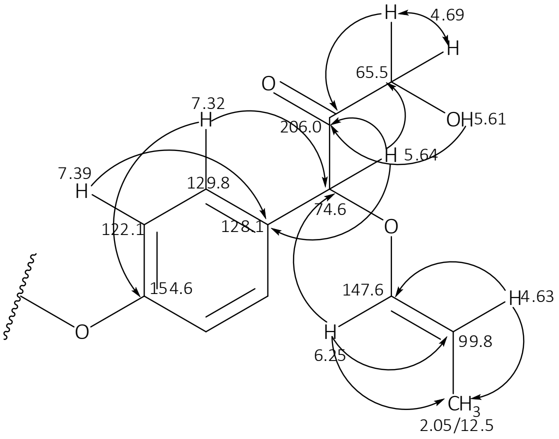

Figure 13: The fifth fragmentation pathway of the LGF-02 molecule.

The combination of these last two fragmentations allowed the determination of the second sequence of the LGF-02 compound molecule.

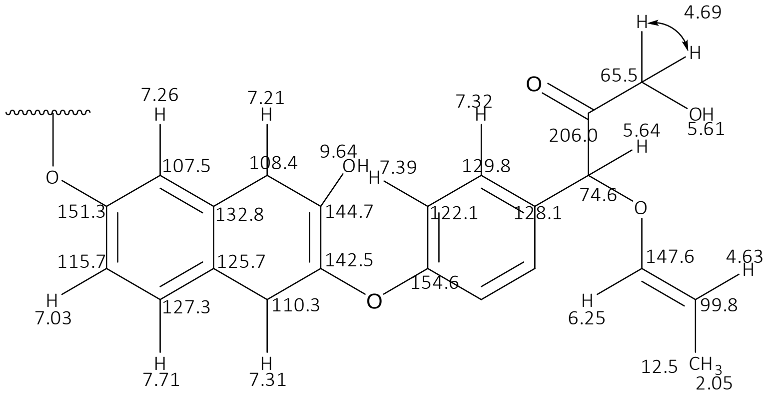

Figure 14: The second structural sequence of the LGF-02 molecule.

The combination of the two sequences leads to the complete molecular structure of the compound LGF-02.

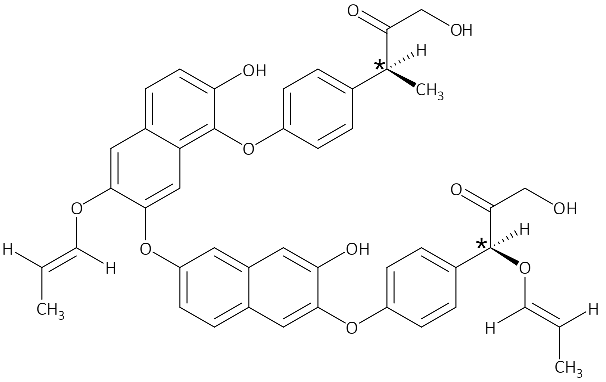

Figure 15: The complete molecular structure of the LGF-02.

3. DISCUSSION

The plant commonly known by the vernacular names **sefana**, **voambanany**, or **mafatamboa** in the southeastern region of Madagascar is well known for its medicinal properties and is used by the local population to kill animals. Following biological screenings, the hydroalcoholic extract exhibited a toxic effect with an LD_{50} of 115 µg/mL. These results support the traditional use of this plant. The genus to which this plant belongs is widespread in West Africa, particularly in the sub-Saharan region, and it is used in folk medicine to treat various diseases such as conjunctivitis, amenorrhea, diabetes, renal insufficiency, and hepatitis.[3] The plant demonstrates antibacterial and anticonvulsant properties[16], indicating its significant role in traditional medicine.

The application of bioassay-guided fractionation enabled the isolation of two pure compounds; however, their biological activities could not be identified due to insufficient quantities. Their complete chemical structures were elucidated using one-dimensional and two-dimensional NMR spectral methods. Literature research on these molecules demonstrated that these two compounds have not been previously reported and are likely novel. Moreover, this is the first time such molecules have been isolated from the species *Cnestis polyphylla*.

4. CONCLUSION

Cnestis polyphylla (Connaraceae) is widely distributed in Madagascar. Due to its proven toxicity, this plant is exploited solely for the purpose of killing rats and stray dogs in villages. Extensive chemical studies on the leaves of *Cnestis polyphylla* led to the isolation of two compounds potentially responsible for the plant's toxicity. Structural elucidation by 1D and 2D NMR spectroscopy resulted in the determination of the structures of these two novel molecules.

All these findings led us to conclude that *Cnestis polyphylla* may contain new bioactive molecules with potential applications in therapy and human medicine.

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