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Triterpenes and antimicrobial activity evaluation of *Cladogelonium madagascariense* Leandri (Euphorbiaceae)

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Abstract

Background: *Cladogelonium madagascariense* Leandri is a single genus, single species endemic plant of Madagascar. The aerial parts are used to treat the fevers with grippe symptoms and neurological signs by steam bath. The aim of this study was to evaluate its antimicrobial activity, isolate and identify its phytochemical constituents.

Methods: The aerial parts of the plant were collected, shade-dried, and pulverized into a fine powder. Preliminary phytochemical screening of the crude ethanol extract was conducted using standard qualitative methods. The antimicrobial activity was evaluated using the disk-diffusion method with the ethanolic extract. Sequential extraction of aerial parts powder was performed by maceration using solvents of increasing polarity: hexane, ethyl acetate, and methanol. Chromatographic separation of the extracts was performed, and compounds isolated from the hexane extract were identified using nuclear magnetic resonance (NMR) spectroscopy and by comparison with literature data.

Results: The screening phytochemical analysis of *Cladogelonium madagascariense* Leandri revealed the presence of flavonols, leucoanthocyanins, triterpenes, steroids, unsaturated sterols, tannins, and saponins in ethanolic extract. Antibacterial study of this species revealed that was active against the six Gram-positive and Gram-negative bacteria: *Bacillus cereus, Bacillus megaterium, Listeria monocytogenes, Enterobacter cloacae, Salmonella enteritidis* and *Streptococcus pneumoniae* using disc diffusion method at the concentration of 2 mg/disc. Fractionation of the hexane extract led to the isolation of five known triterpenes: lupeol (**1**); glut-5-en-3α-ol (**2**); friedelin (**3**); alstrinine (**4**) and hederagenin (**5**).

Conclusion: These findings demonstrate the presence of tetracyclic triterpenes in the hexane and ethyl acetate extracts of *Cladogelonium madagascariense* aerial parts. The ethanol extract exhibit antimicrobial activity. These results highlight the potential of this plant for use in phytomedicine.

Keywords: *Cladogelonium madagascariense;* Antimicrobial; Triterpenes; NMR

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1. Introduction

Cladogelonium madagascariense Leandri (Euphorbiaceae), commonly known by local name "tsontso" is a plant traditionally used in Malagasy medicine. The aerial parts are used to treat the fevers with grippe symptoms and neurological signs by steam bath [1]. Previous studies on the aerial parts of *Cladogelonium madagascariense* showed the anti-inflammatory activity of the extracts and the isolated compound glut-5-en-3α-ol [2],[3],[4].

In this study, we report the antimicrobial activity of ethanol extract, then the phytochemical investigation, isolation, and structural elucidation of isolated compounds from the hexane extract of *Cladogelonium madagascariense* aerial parts.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

The aerial parts of *Cladogelonium madagascariense* Baker were collected in Fanambana, Vohemar District, SAVA Region, Madagascar, in March 2022. The plant specimen was identified by a botanist at the Botanical and Zoological Park of Tsimbazaza (PBZT). The aerial parts were dried, ground into a fine powder, and stored in an airtight container for future use.

2.1.2. General experimental procedures

All organic solvents were distilled prior to use. Extracts were fractionated by liquid chromatography on silica gel (Merck MN Silica Gel 60 M; particle size: 0.04–0.063 mm), eluted under atmospheric pressure. NMR data, including ^{1}H, ^{13}C, DEPT, ^{1}H–^{1}H COSY, ^{1}H–^{13}C HSQC, and ^{1}H–^{13}C HMBC, were recorded in deuterated solvents (CD_3OD, $CDCl_3$, or DMSO) using a Bruker 600 MHz or 300 MHz NMR spectrometer (operating at 600.19 MHz/300 Mhz for ^{1}H and 125.78 MHz for ^{13}C). Chemical shifts (δ) are reported in parts per million (ppm), using tetramethylsilane (TMS) as the internal standard. The structures of isolated compounds were confirmed by comparison of their spectroscopic data with literature values. Thin-layer chromatography (TLC) was performed on aluminum-backed silica gel plates (Macherey-Nagel, SIL G/UV254, 0.20 mm). Spots were visualized under UV light at 254 and 366 nm, or by heating after spraying with a 20% (v/v) sulfuric acid solution.

2.1.3. Preparation of extracts

The dried and powdered aerial parts (50 g) were extracted with EtOH 80° (220 mL) by maceration for 7 days at room temperature, resulting in the crude ethanolic extract after evaporation under reduced pressure of the solvent. In order to separate the chemical families by polarity, extractions using solvents by increasing polarities were made, the dried and powdered aerial parts (400 g) were macerated successively in solvents of increasing polarity: hexane (1250 mL for 10 days), ethyl acetate (1250 mL for 3 days), and methanol (1250 mL for 3 days). The resulting solutions were filtered and concentrated under reduced pressure to yield three extracts: hexane, ethyl acetate, and methanol.

2.1.4. Phytochemical screening

Phytochemical screening was performed with ethanolic extract as previously described, using specific reagents to qualitatively assess the presence of various compounds [5]. These included alkaloids, iridoids, steroids, triterpenoids, saponins, polysaccharides, and polyphenols such as coumarins, flavonoids, leucoanthocyanins, and tannins.

2.1.5. Antimicrobial assay

The crude ethanolic extract of *Cladogelonium madagascariense* aerial parts was tested for antimicrobial activity against five Gram-positive bacterial strains : *Streptococcus pneumoniae* ATCC 6301, *Staphylococcus aureus* ATCC 11632, *Bacillus cereus* ATCC 13061, *Listeria monocytogenes* ATCC 700324, *Bacillus megaterium* ATCC 13062, against four Gram-negative bacterial strains : *Klebsiella oxytoca* ATCC 8724, *Enterobacter cloaceae* ATCC 700323, *Salmonella enteritidis* ATCC13076, *Escherichia coli* ATCC 25922 and one fungal strain: *Candida albicans* ATCC 10231, by a previously described disk diffusion method [6], in Petri dishes. Sterile disc of 6 mm in diameter (Biomérieux, Marcy l'Etoile, France) impregnated with 20 µL of extract with a concentration equal to 100 mg /mL or 2 mg / disc are placed on the surface of the seeded agars. The petri dishes are then incubated at 37 °C. The diameter of the inhibition zone (mm) around each disc is measured after 24 h. The reference antibiotic gentamycin (20 µg / disc), Nystatin (20 µg / disc) and Netilmicin

(30 µg / disc), chloramphenicol (30 µg / disc) [7] are used as a positive control. All tests were performed in triplicate, and clear halos greater than 7 mm were considered as positive results [8].

2.1.6. Fractionation and isolation

The hexane extract (2.5 g) was subjected to column chromatography on silica gel (80 g of silica gel 60, column dimensions 50 × 3.5 cm), using a gradient elution of hexane and ethyl acetate, affording 550 fractions of 10 mL each. Fractions with similar TLC profiles were combined and subjected to purification by crystallization. This led to the isolation of five compounds: 1 (8.1 mg, white powder), 2 (41.7 mg, white powder), 3 (41.7 mg, white powder), 4 (12 mg, white powder), and 5 (10 mg, whitish powder) obtained from fractions 101–110 (eluted with hexane/ethyl acetate 98:2), 120–129 (hexane/ethyl acetate 98:2), 150–155 (hexane/ethyl acetate 98:2), 288–292 (hexane/ethyl acetate 60:40) and 508–520 (hexane/ethyl acetate 10:90) respectively.

The structures of the isolated compounds were elucidated by spectroscopic analysis and by comparison of their NMR data with those reported in the literature.

3. Results

3.1. Preparation of extracts

Maceration in EtOH 80° of 50 g of the plant material yielded 6.6 g (13.2%) of crude ethanolic extract. For extraction with the solvent of increasing polarity, with 400 g of dry powder, 4,4 g (1,10 %) of hexanic extract; 6,1 g (1,53 %) of ethyl acetate extract and 7,2 g (1,80 %) of methanolic extract were obtained.

3.2. Preliminary phytochemical screening

The screening phytochemical analysis of *Cladogelonium madagascariense* aerial parts revealed the presence of flavonols, leucoanthocyanins, triterpenes, steroids, unsaturated sterols, tannins, and saponins, while alkaloids and coumarins were absent.

3.3. Assay of antimicrobial activity

The antibacterial and antifungal activities of the crude ethanol extract, of *Cladogelonium madagascariense* Leandri aerial parts are shown in the table 1.

Table 1 Antimicrobial assay of the crude ethanolic extract of *Cladogelonium madagascariense* Leandri.

Sample	Diameter of inhibitory zone (mm)				
	Crude ethanolic	**Gentamycin 20 µg**	**Nystatin 20 µg**	**Netilmicin 30 µg**	**Chloramphenicol 30 µg**
Streptococcus pneumonia	18± 0.2	+++	nt	23	25
Staphylococcus aureus	6± 0.2	++++	nt	17	30
Bacillus cereus	7± 0.2	+++	nt	22	38
Listeria monocytogenes	10± 0.2	nt	nt	nt	30
Bacillus megaterium	8± 0.2	nt	nt	24	nt
Enterobacter cloaceae	12± 0.2	nt	nt	nt	32
Salmonella enteritidis	6± 0.2	++++	nt	nt	30
Escherichia coli	10± 0.2	++	nt	nt	25
Klebsiella oxytoca	6± 0.2	nt	nt	nt	nt
Candida albicans	7± 0.2	nt	+++	nt	nt

(++): Inhibition diameter between 11–15 mm; (+++): Inhibition diameter between 16–20 mm;
(++++): Inhibition diameter higher than 20 mm; nt: no tested.

The ethanolic extract showed antibacterial activity against the six bacterial strains with diameters of inhibition zones ranging between 7.0 ± 0.2 and 18 ± 0.2 mm at a concentration of 2 mg/disc : *Bacillus cereus* (7± 0.2 mm), *Bacillus megaterium* (8 ± 0.2mm), *Listeria monocytogenes* (10± 0.2 mm), and *Enterobacter cloacae* (10± 0.2 mm). It was also noted to be effective against *Salmonella enteritidis* (12± 0.2 mm) and *Streptococcus pneumoniae* (18± 0.2 mm). But it's showed no activity against the strains of *Staphylococcus aureus*, *Escherichia coli, Klebsiella oxytoca*, and *Candida albicans* (inhibition diameter < 7 mm).

3.4. Spectral data and identification of isolated compounds

The structures of the isolated compounds were determined through comprehensive analysis of their ^{1}H, ^{13}C, DEPT, HSQC, and HMBC NMR spectra, and by comparison with previously reported data in the literature. Compounds **1, 2, 3**, **4,** and **5** were identified as lupeol (**1**) [9], glut-5èn-3-ol (**2**) [2],[10], friedelin (**3**) [11], alstrinine (**4**) [12] and hederagenin (**5)** [13] respectively. The chemical structures of these compounds are presented in Figure 1.

Lupeol (**1**): δ (ppm)^{1}H NMR (600.19 MHz, $CDCl_3$): 4.62(1H, s, H-29)), 4.50(1H, s, H-29), 3.12(1H, dd, H-3), 2.30(1H, m, H-19), 1.61(3H, s, H-30), 0.96(3H, s, H-26), 0.90(3H, s, H-24), 0.87(3H, s, H-27), 0.76(3H, s, H-25), 0.73(3H, s, H-28), 0.69(3H, s, H-23). δ (ppm)^{13} C NMR (125.78 MHz, $CDCl_3$): 150.8 (C-20), 107.0 (C-29), 79.1 (C-3), 55.2 (C-5), 50.2 (C-9), 48.2 (C-,19), 47.4 (C-18), 42.6 (C-14), 42.5(C-17), 41.0 (C-8), 39.5 (C-22), 38.9(C-13), 38.8(C-4), 38.0 (C-1), 37.0 (C-10), 36.4 (C-15), 35.5 (C-16), 34.7 (C-7), 29.6 (C-21), 28.0 (C-24), 27.4 (C-12), 26.0 (C-2), 21.0 (C-30), 20.9 (C-11),18.0 (C-6),17.9 (C-28), 17.6 (C-23), 15.7 (C-25), 15.1 (C-26), 14.2 (C-27).

Glut-5-en-3α-ol (**2**): δ(ppm) ^{1}H NMR (600.19 MHz, CD_3OD): 0.85 (3H, s, H-25), 0.95 (3H, s, H-29), 0.99 (3H, s, H-30), 1.00 (3H, s, H-27), 1.04 (3H, s, H-23), 1.09 (3H, s, H-26), 1.14 (3H, s, H-24), 1.16 (3H, s, H-28), 3.49 (1H, d, H-3), 5.62 (1H, t, H-6). δ (ppm) ^{13}C NMR (CD_3OD, 125.78 MHz): 16.2 (C-25), 18.2 (C-1), 18.4 (C-27), 19.7 (C-26), 23.6 (C-7), 25.4 (C-24), 27.8 (C-2), 28.3 (C-20), 28.9 (C-23), 30.1 (C-17), 30.4 (C-12), 32.0 (C-28), 32.1 (C-15), 32.4 (C-30), 33.1 (C-21) , 34.4 (C-29), 34.5 (C-11), 34.8 (C-9), 35.1 (C-19), 36.0 (C-16), 37.8 (C-13), 38.8 (C-22), 39.4 (C-14), 40.8 (C-4), 43.0 (C-18), 47.5 (C-8), 49.6 (C-10), 76.3 (C-3), 122.0 (C-6), 141.7 (C-5).

Friedelin (**3**): δ(ppm) ^{1}H NMR (600.19 MHz, CD_3OD): 0.73 (3H, s, H-24), 0.85 (3H, s, H-25), 0.90 (3H, d, H-23) 0.96 (3H, s, H-29), 1.021 (3H, s, H-30), 1.022 (3H, s, H-27), 1.06 (3H, s, H-26), 1.21 (3H, s, H-28). δ (ppm) ^{13}C NMR (CD_3OD, 125.78 MHz): 6.8 (C-23), 14.7 (C-24), 18.0 (C-25), 18.2 (C-7), 18.7 (C-26), 20.3 (C-27), 22.3 (C-1), 28.2 (C-20), 30.0 (C-17), 30.5 (C-12), 31.8 (C-30), 32.1(C-15), 32.4 (C-28), 32.8 (C-21), 35.0 (C-29), 35.3 (C-11) , 35.6 (C-19), 36.0 (C-16), 37.4 (C-9), 38.3 (C-14), 39.3 (C-22), 39.7 (C-13), 41.3 (C-6), 41.5 (C-2), 42.2 (C-5), 42.8 (C-18), 53.1 (C-8), 58.2 (C-4), 59.5 (C-10), 213.2 (C-3).

Alstrinine (**4**): δ (ppm) ^{1}H NMR ($CDCl_3$, 300 MHz): 5.03 (1H, s, H-12), 5.02 (1H, s, H-6), 4.63 (1H, s, H-29), 4.50 (1H, s, H-29), 3.17 (1H, dd, H-22), 3.16 (1H, dd, H-3), 2.30 (1H, m, H-19), 1.61(3H, s, H-30), 0.77 (3H, s, H-26), 0.96 (3H, s, H-24), 0.90 (3H, s, H-27), 0.87 (3H, s, H-25), 0.78 (3H, s, H-28), 0.90 (3H, s, H-23).

Hederagenin (**5**): δ (ppm) ^{1}H NMR (CD_3OD, 600 MHz): 0.53 (3H, s, H-23), 0.74(3H, s, H-26), 0.88 (3H, s, H-25, 29, 30), 1.10(3H, s, H-27), 3.08 (1H, d, H-24), 3.40(1H, H-3), 5.15(1H, t, H-12). δ (ppm) ^{13}C NMR (CD_3OD, 125.78 MHz): 12.9 (C-23), 15.9 (C-25), 17.3 (C-26), 17.9 (C-6), 23.0 (C-16), 23.3 (C-11), 24.0 (C-29), 26.3 (C-27), 26.9 (C-2), 27.3 (C-15), 30.7 (C-20), 32.3 (C-7), 32.5 (C-22), 33.2 (C-30), 33.7 (C-21), 36.7 (C-10), 37.0 (C-1), 38.4 (C-8), 41.2 (C-18), 41.6 (C-14), 42.2 (C-4), 45.9 (C-19), 46.1 (C-17), 46.8 (C-5), 47.5 (C-9), 64.7 (C-24), 70.7 (C-3), 122.1 (C-12), 144.5 (C-13), 179.1 (C-28).

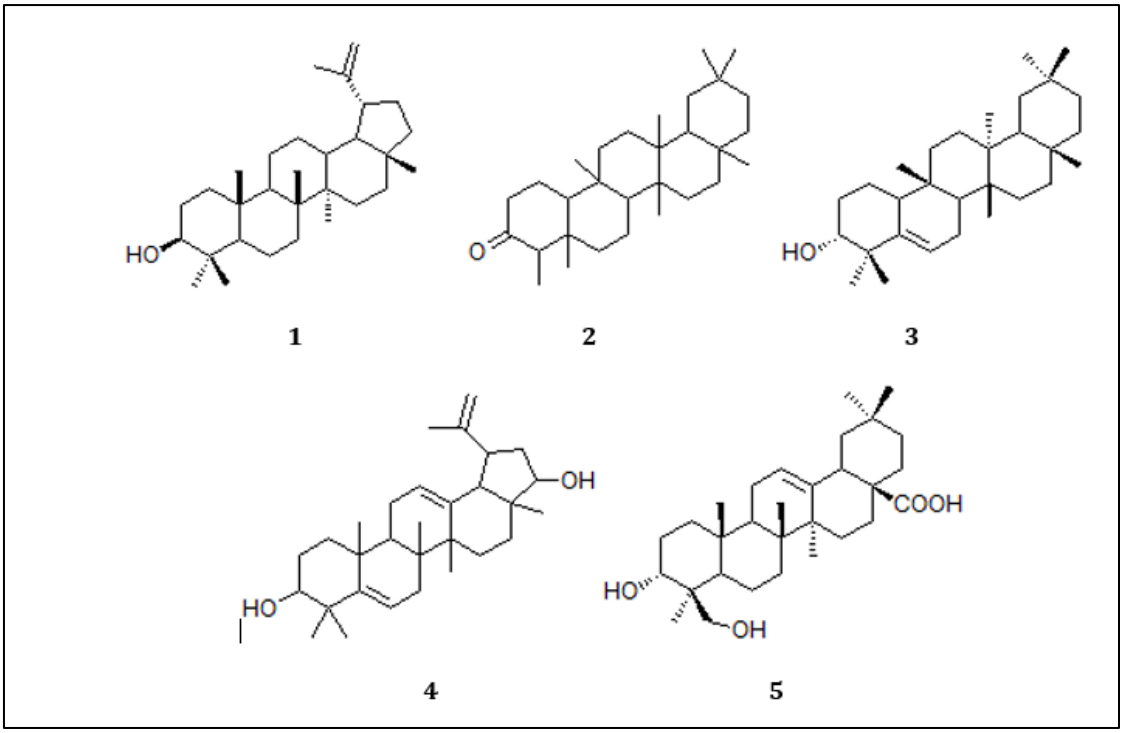

Figure 1 Structures of isolated compounds from *Cladogelonium madagascariense* Leandri: lupeol (**1**), glut-5-èn-3α-ol (**2**), friedelin (**3**), alstrinine (**4**), and hederagenin (**5**)

4. Discussion

This study on *Cladogelonium madagascariense* reports the isolation of five pentacyclic triterpenes from the aerial parts: lupeol (**1**), glut-5-en-3-ol (**2**), friedelin (**3**), alstrinine (**4**), and hederagenin (**5**). This study presents the first isolation of four with five isolated triterpenes from the aerial parts of *Cladogelonium madagascariense*, contributing valuable insights into the phytochemical profile of this monospecies and monogenus plant endemic to Madagascar. Lupeol (**1**), friedelin (**3**), and glut-5-en-3α-ol (**2**), on the other hand, have previously been reported in *Euphorbia convolvuloides* [14], *Euphorbia kamerunica* [15], and *Euphorbia pseudocactus* [16], respectively. The identification of glut-5-en-3α-ol, friedelin, and lupeol reinforces the classification of *Cladogelonium madagascariense* within the Euphorbiaceae family, aligning with previous taxonomic assessments. Notably, the discovery of hederagenin and alstrinine expands the known chemical diversity of the Euphorbiaceae, as these compounds have not been previously reported within this family, despite the presence of a saponoside derived from hederagenin in *Euphorbia paralais* [17]. The antimicrobial activity of ethanolic extract against *Bacillus cereus*, *Bacillus megaterium*, *Listeria monocytogenes*, *Enterobacter cloacae*, *Salmonella enteritidis* and *Streptococcus pneumoniae* at the concentration of 2 mg/disc of *Cladogelonium madagascariense*, evaluated for the first time in this study, highlights the potential of this plant as a source of bioactive compounds. Diverse antimicrobial activities have been described in literature for some isolated compounds. Indeed, friedelin has demonstrated antibacterial activity against *Pseudomonas aeruginosa, Salmonella typhii* and *Staphylococcus aureus* [15], while hederagenin has an antibacterial activity against *Streptococcus pneumoniae* [18]. The presence of hederagenin and friedelin, both recognized for their antimicrobial properties, could be responsible for antimicrobial effects observed of ethanolic extract. This finding opens avenues for further research into the therapeutic potential of *Cladogelonium madagascariense* in the context of developing natural antimicrobial agents.

5. Conclusion

This study presents the first antimicrobial investigation of the ethanolic extract of *Cladogelonium madagascariense* Leandri. The aerial parts were found to contain tetracyclic triterpenes lupeol, glut-5-en-3α-ol, friedelin, alstrinine and hederagenin. Importantly, four of the five isolated compounds are reported here for the first time from *Cladogelonium madagascariense*, highlighting the novelty of this research.

The results provide a scientific basis for the traditional use of *Cladogelonium madagascariense* aerial parts in Malagasy medicine and indicate its potential value in antimicrobial activity.

Compliance with ethical standards

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Disclosure of conflict of interest

No competing financial interests to declare.

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