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(RESEARCH ARTICLE)

Phytochemical investigation of the bark extract of *Carissa sessiliflora Brongn. Ex Pichon Syn. Carissa bispinosa (L) Desf.ex Brenan* (Apocynaceae), endemic to Madagascar

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Abstract

Carissa sessiliflora Brongn. ex Pichon Syn. Carissa bispinosa (L) Desf.ex Brenan (Apocynaceae) is endemic to western Madagascar. Due to its therapeutic properties, this plant plays an important role in traditional medicine and is used to treat infections and malaria.

Phytochemical investigations of the cyclohexane extract of this plant's stem bark have led to the isolation of four pure compounds. The chemical structures of these compounds were elucidated using spectroscopic analysis techniques (1D and 2D NMR) and mass spectrometry (ESI-MS in positive mode), as well as by comparing the data with that in the literature.

These products are attributed to the chemical family of pentacyclic triterpene compounds.

Keywords: *Carissa sessiliflora Brongn* ex. *Pichon*; Apocynaceae; Pentacyclic triterpene; NMR (1D, 2D) and EI.MS

1. Introduction

The genus *Carissa belongs* to the Apocynaceae family and comprises around 35 species of evergreen trees and shrubs, each more fascinating than the last. Most of these species are found in tropical and subtropical regions [1]. According to the literature, more than 30 species of this genus have been recorded in tropical Africa, Asia and Australia, and several of these species are used in traditional herbal medicine [2-6].

Carissa sessiliflora Brongn. ex *Pichon*, which is synonymous with *Carissa bispinosa (L.) Desf. ex Brenan*, is one of the species that are endemic to Madagascar. On the Big Island, it is known by the vernacular names Voatsokopika (Sakalava language) and Hazoambo (Bezanozano language). This plant is well known in western Madagascar for its therapeutic properties in traditional medicine. Ethnobotanical surveys conducted in the districts of Morondava, Mahabo, Manja and Belotsiribihina in western Madagascar revealed that the leaves are used to treat malaria, chest pain and chronic coughs, while the bark from the stems and roots is used to treat low libido, impotence and tuberculosis.

No advanced scientific studies on this plant have been identified in bibliographic studies.

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These findings prompted us to conduct in-depth phytochemical studies on extracts from the bark and stems of *Carissa sessiliflora Brongn.* ex *Pichon*. This study aims to identify and isolate the terpenic chemical family present in Voatsokopika bark extract using chromatography. The plant was collected in Kirindy Forest in the rural commune of Befasy in the Morondava district of the Menabe region in western Madagascar.

2. Material and method

2.1. General

Melting points were measured using a Buchi 510 apparatus and are uncorrected. IR data were recorded using a Perkin-Elmer 1710 spectrophotometer. The 1H NMR (300 and 400 MHz) and 13C NMR (75 and 100 MHz) spectra were measured on a Bruker AM 300 spectrometer and a Bruker AM 400 spectrometer, with TMS as the internal standard. EIMS data were obtained using a Finnigan MAT 312 spectrometer. Chromatographic separations were performed by column chromatography (CC) using silica gel 60 (70-230 mesh, Merck) or Sephadex LH-20 (Pharmacia). Analytical TLC was performed on Merck Kieselgel 60 F254 plates with a film thickness of 0.2 mm. Spots on the TLC were then visualised by UV light or by spraying with a reagent based on anisaldehyde and sulfuric acid, followed by heating at 120 °C.

2.2. Plant material

The bark of the stems of the plant known by the vernacular name "Voatsokopika" (Sakalava name) was collected in the Kirindy forest, in the rural commune of Befasy, in the district of Morondava, in the Menabe region, in western Madagascar, in March 2022. Mr. Benja Rakotonirina, a botanist at the Malagasy Institute of Applied Research, identified this plant as *Carissa sessiliflora Brongn*. ex *Pichon*, syn. *Carissa bispinosa (L.) Desf. ex Brenan*. It belongs to the Apocynaceae family. The herbarium specimen of this plant has been deposited at the herbarium of the Malagasy Institute of Applied Research, Avarabohitra Itaosy lot AVB 77, P.O. Box 3833, 102 Antananarivo, Madagascar.

2.3. Extraction

820 g of *Carissa sessiliflora Brongn*. ex *Pichon* stem bark was dried and then ground into a powder using a mechanical grinder. This powder was cold macerated in ethanol, under constant stirring, three times for four hours at room temperature in the laboratory. After filtration, the solutions obtained were evaporated to dryness, then dried using anhydrous sodium sulfate, yielding a crude extract designated ET-CS (74.85 g). Sixty grams of this extract were dissolved in demineralized water, and the aqueous solution obtained was then subjected to partition extraction with cyclohexane, ethyl acetate, and n-butanol. The solutions obtained were evaporated to dryness and dried using anhydrous sodium sulfate, yielding four extracts: E-CycloHex (16.3 g), E-ACoET (19.5 g), E-nBut (17.6 g), and E-Aq (7.6 g).

2.4. Isolation

15 g of the cyclohexane extract were fractionated using a silica gel chromatography column (CC), using a mixture of petroleum ether and ethyl acetate to remove the compounds from this column. Ten fractions (FI-FX) were obtained at the end of this column. The isolation process was continued from fraction FIII. This fraction was separated using a low-pressure silica gel chromatography column, eluted with a mixture of chloroform and methanol (9/1) in isocratic mode. At the end of this operation, six fractions (FIII1-FIII6) were obtained. Thin-layer chromatography analysis of fraction FIII4 revealed that it was not pure. Fraction FIII4 was then fractionated further on a Sephadex LH-20 gel chromatography column, eluted with a mixture of dichloromethane and methanol (1:1), yielding four fractions. Following thin-layer chromatography analysis, these four fractions show a single spot, indicating that they are likely to be pure products. To purify them, these four fractions were subjected to preparative chromatography, and four pure products were isolated: product 1 (33 mg), compound 2 (102 mg), compound 3 (32 mg), and compound 4 (28 mg).

2.5. Anti-malarial bioassay [7-14]

2.5.1. Parasite's strain and in vitro culture conditions

The asexual erythrocytes' stages of Plasmodium falciparum FcM29-Cameroon, a highly chloroquine-resistant strain were grown continuously in stock cultures by a modification of the methods of Trager and Jensen using glucose enriched RPMI 1640 medium, supplemented with 10% human serum at 37 °C as previously reported.

2.5.2. In vitro antiplasmodial activity

The antiplasmodial activity of the plant extracts was evaluated by an isotopic micro-test which determines the inhibition of radio labeled hypoxanthine up take by malaria parasite as an indicator of growth.

2.5.3. Test extract preparation

Methanol (MeOH, 200 µL) or dimethylsulfoxide (DMSO) was added to 1 mg sample of extracts and further diluted as required in water. The MeOH or DMSO concentration for tested dilutions was not greater than 1%. Initial concentration of the plant extracts was 50 µg/mL diluted with five-fold dilutions to make five concentrations, the lowest being 0.08 µg/mL. Each test included an untreated control with the solvent and a positive control: chloroquine sulphate (Sigma, France) and ethanolic crude extract of Cinchona stem bark.

2.5.4. Isotopic micro-test

Two hundred micro liters (200 µL) of total culture medium with the diluted extract (20 µL) and the suspension (180 µL) of Plasmodium falciparum-infected human red blood cells in medium (O+ group, 1% haematocrit) with 1% asynchronous parasitaemia was placed into the wells of 96-well micro titre plates. After 18 h incubation of the parasites with the extracts at 37 °C, [3H] hypoxanthine (Amersham, UK) was added to each well and the incubation was continued for another 24 h at the same conditions. The mean values for uptake of 3H-hypoxanthine (disintegration per minute, dpm) in control (untreated) and tested parasitized erythrocytes, expressed as the percentage of inhibition, were calculated. The anti-malarial activity of extracts was expressed by the inhibitory concentrations 50% (IC50), representing the concentration of drug that induced 50% parasitemia decrease compared to control culture. The extract concentration at which the parasite growth (ie [3H] hypoxanthine uptake) is inhibited by 50% (IC50) was calculated by a non-linear regression analysis processed on dose–response curves with the help of Mikro Win Hidex 2000 software. Liquid scintillation counting was operated on CHAMELEONTM V multilabel counter plate.

2.5.5. In vivo antiplasmodial activity

The in vivo anti-malarial activity of plant extracts was determined by the classical 4-day suppressive test against Plasmodium yoelii subsp nigeriensis strain.

2.5.6. Preparation of parasite inoculum

Five albino Swiss mice of 6 to 8 weeks old and weight ranging between 19 and 30 g were inoculated by intravenous route with 200 µL of the parasite suspension. On day 3, a blood smear was carried out for each mouse. Only the blood of the mice having presented a parasitemia between 30 and 50% was used as inoculum.

2.5.7. In vivo antiplasmodial assay

To realize the test, 25 male Swiss albino mice were inoculated by intravenous route with 107 Plasmodium yoelii infected red blood cells (parasite inoculum) and randomly divided into five groups of five mice per cage. Chloroquine (CQ) and 3% Tween 80 were used as positive and negative control respectively. The test extracts were prepared in three different doses (200 mg/kg, 400 mg/kg, and 600 mg/kg of body weight) and CQ at 25 mg/kg in a volume of 0.2 mL and vehicles at 0.5 mL/mouse. Each extract was administered as a single dose per day. All the extracts and the drug were given through intragastric route by using standard intragastric tube to insure safe ingestion of the extracts and the drug. Treatment was started after 3 hrs of infection on day 0 and was then continued daily for four days (i.e. from day 0 to day 3). On the fifth day (D4) blood sample was collected from tail snip of each mouse. Thin smears were prepared and stained with 10% Giemsa solution. Then, each stained slide was examined under the microscope with an oil immersion objective of 100× magnification power to evaluate the percent suppression of each extract with respect to the control groups. All experimental procedures were approved by the Malagasy Institute of Applied Research (IMRA) Avarabohitra- Itaosy lot AVB 77, P.O. Box 3833, 102 Antananarivo- Madagascar.

2.6. Statistical analysis

The results of *in vitro* study are given as Mean ±S tandard Deviation obtained from three independent experiments. The results of in vivo study were expressed also as Mean ± Standard Deviation and analyzed with Student's t-test for paired data using Origin version 8.5 Pro package software. All data were analyzed at a 95% confidence interval (α= 0.05).

3. Results and discussion

Samples of bark were collected from the stems of the plant, dried, and ground into a powder using a mechanical grinder. 820g of the plant matrix powder were extracted with absolute ethanol. The resulting ethanol extract was transferred to demineralized water and partitioned using cyclohexane, ethyl acetate, and n-butanol. Four extracts were obtained: the cyclohexane extract (E-CycloHex), the ethyl acetate extract (E-ACoET), the n-butanol extract (E-nBut), and the aqueous extract (E-Aq).

A series of chromatographic analyses were performed on the cyclohexane extract, including low-pressure open column chromatography on silica gel and Sephadex LH-20 gel, as well as thin-layer and preparative chromatography. Four pure products were isolated. We established the chemical structures of these compounds (fig.1) by interpreting NMR (1D and 2D) and ESI-MS (70 eV in positive mode) spectroscopic analysis data.

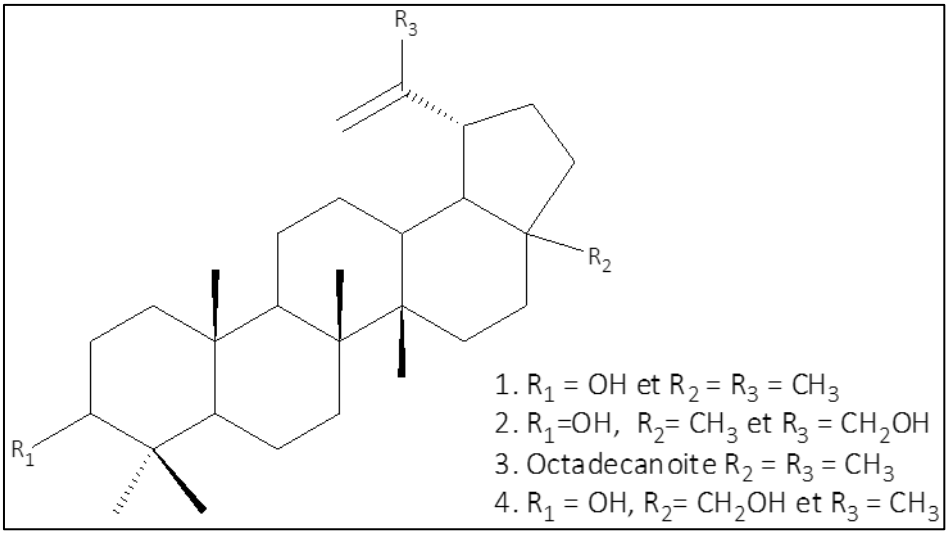


Figure 1 The chemical structures of the four isolated molecules

A comparative study of the spectral data of these isolated products and literature databases determined that all four compounds belong to the pentacyclic triterpene family: (1) 3β-hydroxylup-20(29)-ene, (2) 3β,30-dihydroxylup-20(29)-ene, (3) lupeol 3-dihydroxyoctadecanoate, and (4) 3β,28-dihydroxylup-20(29)-ene.

Through bibliographic research, we discovered that these molecules are all described in the literature and are known, respectively, as lupeol [15-19], hennadiol [20-24], procrim B [25], and betulin [26-28]. These molecules have never before been isolated from the bark of Carissa sessiliflora stems.

In terms of biological activity, the crude stem bark extract exhibits antimalarial effects in vivo and *in vitro*, with respective inhibitory concentrations of 5.42 ± 0.618 mg/kg (n = 6) and 1.113 ± 0.084 µg/mL (n = 6). After separating the crude extract, four extracts were obtained: E-CycloHex, E-ACoET, E-nBut, and E-Aq. However, the antimalarial tests performed on these four extracts show that none of them have antimalarial effects, either in vivo or *in vitro*. When these four extracts were combined and tested, they exhibited promising antimalarial activity. These results prove that the antimalarial activity of Carissa sessiliflora bark extract is due to the synergistic action of two or more molecules present in the extract. These results also corroborate ethnobotanical data regarding this plant [11, 13].

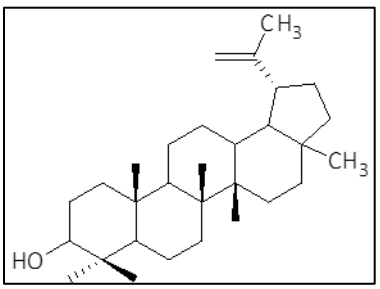
The data pertaining to the isolated products, obtained through the utilization of NMR spectroscopy (1D and 2D) and mass spectrometry (IE-MS, 70eV in positive mode), is appended herein.

3.1. Compound.1

^{1}H-NMR (300 MHz, $CDCl_3$): δ (ppm)= 4.69 (1H, d, J= 1.2 Hz, H-29a), 4.56 (1H, d, J= 1.2 Hz, H-29b), 3.20 (1H, dd, H-3), 2.39 (1H, ddd, J= 11.0, 11.0, 5.5 Hz, H-19), 1.68 (3H, s, H_3- 30), 1.03 (3H, s, H_3-26), 1.97 (3H, s, H_3-23), 0.95 (3H, s, H_3-27), 0.83 (3H, s, H_3-25), 0.79 (3H, s, H_3-28), 0.76 (3H, s, H_3-24).

^{13}C-NMR (75 MHz, $CDCl_3$): δ (ppm)= 150.9 (C-20), 109.3 (C-29), 78.9 (C-3), 55.3 (C-5), 50.4 (C-9), 48.3 (C-18), 47.9 (C-19), 42.9 (C-17), 42.8 (C-14), 40.8 (C-8), 39.9 (C-22), 38.8 (C-4), 38.7 (C-1), 38.0 (C-13), 37.1 (C-10), 35.5 (C-16), 34.3 (C-7), 29.8 (C-21), 27.9 (C-23), 27.4 (C-15, 2), 25.1 (C-12), 20.9 (C-11), 19.3 (C-30), 18.3 (C-6), 18.0 (C-28), 16.0 (C-25), 15.9 (C-26), 15.3 (C-24), 14.5 (C-27).

EI-MS (70 eV) m/z (rel. Int.)= 426 (M^+, 100). 393 (3), 316 (9), 315 (8), 219 (11), 218 (39),205 (12), 204 (15), 203 (19), 191 (16), 189 (31), 149 (15), 135 (23), 123 (14), 109 (21), 107 (17), 95 (18), 85 (32), 69 (12).



Compound.1 : [3β-Hydroxylup-20(29)-ène]

Gross formula : $C_{30}H_{50}O$

PF : 208-210°C

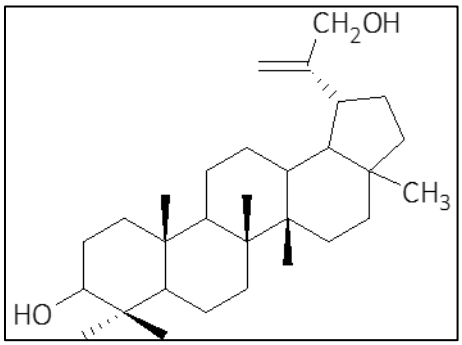
Molecular ion at m/z : 426

3.2. Compound.2

^{1}H-NMR (400 MHz, Pyridin): δ(ppm)= 6.60 (1H, s, OH), 5.88 (1H, s, OH), 5.47 (1H, s, H-29a), 5.10 (1H, s, H-29b), 3.44 (1H, H-3), 2.45 (1H, m, H-19), 2.12 (1H, m, H-21a) , 1.87 (1H, m, H-2a), 1.85 (1H, m, H-2b), 1.65 (2H, m, H-1a,15a), 1.62 (1H, m, H-12a), 1.61 (1H, m, H-18), 1.59 (2H, m, H-12b,15b), 1.58 (1H, H-13), 1.55 (1H, m, H-6a), 1.54 (1H, m, H-21b), 1.50 (1H, m, H-16a), 1.41 (1H, m, H-22b), 1.40 (2H, m, H-6b,16b), 1.38 (2H, ,m, H-7),1.34 (1H, m, H-22b), 1.30 (1H, m, H-11a), 1.26 (1H, dd, H-9), 1.21 (3H, s, H_3-23), 1.11 (1H, m, H-11b), 1.02 (3H, s, H_3-24), 1.01 (3H, s, H_3-26), 0.95 (3H, s, H_3-27), 0.85 (3H, s, H_3-25),0.81 (3H, s, H_3-28), 0.76 (1H, H-5).

^{13}C-NMR (100.5 MHz, Pyridin): δ (ppm)= 156.6 (C-20), 106.5 (C-29), 78.4 (C-3), 64.4 (C-30), 56.1 (C-5), 51.0 (C-9), 49.2 (C-18), 44.4 (C-19), 43.4 (C-14), 43.2 (C-17), 41.4 (C-8), 40.4 (C-22), 39.7 (C-4), 39.5 (C-1), 38.6 (C-13), 37.7 (C-10), 36.0 (C-16), 35.C-7), 32.4 (C-21), 28.9 (C-23), 28.4 (C-2), 28.0 (C-15), 27.1 (C-12), 21.4 (C-11), 19.0 (C-6), 18.1 (C-28), 16.7 (C-25), 16.6 (C-26), 16.5 (C-24), 15.0 (C-27).

EI-MS (70 eV) m/z (rel. Int.)= 442 (M^+, 45), 427 (7), 242 (18), 411 (13), 409 (12), 316 (25),235 (35), 221 (24), 209 (31), 208 (100), 204 (38), 190 (38), 189 (78), 187 (34), 136 (30), 135 (58), 123 (33), 121 (47), 107 (45), 95 (58), 93 (37), 81 (45), 69 (36).



Compound.2: [3β,30-Dihydroxylup-20(29)-ène]

Gross formula : $C_{30}H_{50}O_2$

PF: 236-237 °C.

Rf= 0.49 (Chloroform/Methanol 95/5)

Molecular ion at m/z : 442 [M^+, 45]

3.3. Compound.3

[Lupeol-3-hydroxyoctadecanoate]

Gross formula: $C_{48}H_{84}O_3$

PF : 78-79°C.

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 4.68 (1H, br s, H-29a), 4.56 (1H, br s, H-29b), 4.53 (1H; dd; J= 9.0, 6.6 Hz; H-3), 3.98* (1H, m, H-3′), 2.50* (1H; dd; J= 17.3, 3.3 Hz; H-2′a),2.39 (2H, m, H-2′b, 19), 1.91 (1H, m, H-21a), 1.68 (5H, s, dont H-1a, H3-30), 1.67* (2H), 1.65 (3H, m, H-2a,11, 15), 1.63 (2H, H1a,13), 1.53 (1H, m, H-6a), 1.52 (1H, m, H-16a), 1.50 (2H, m, H-4′a,12b), 1.46* (1H, H-4′b), 1.47 (1H, m H-16b), 1.46* (1H), 1.42 (3H, m, H- 6a,7), 1.40* (2H), 1.38* (1H), 1.36 (2H, m, H-18,21b), 1.34 (1H, m, H-12b), 1.31 (1H, m, H- 9), 1.30* (1H), 1.26* (2H), 1.25* (14H), 1.19 (1H, m, H-22b), 1.10 (1H, m, H-11b), 1.03 (3H, s, H3-26), 1.00 (1H, m, H-15b), 0.94 (3H, s, H3-27), 0.88* (3H, t, J= 5.6 Hz, H3-16′), 0.85 (6H, s, H3-23, H3-25), 0.84 (3H, s, H3-24), 0.82 (1H, H-5), 0.78 (3H, s, H3-28). * (Acid gras)

^{13}C-NMR (100.5 MHz, $CDCl_3$): δ(ppm)= 172.8* (C-1′), 150.9 (C-20), 109.3 (C-29), 81.4 (C-3), 68.2* (C-3′), 55.4 (C-5), 50.3 (C-9), 48.3 (C-18), 48.2 (C-19),43.0 (C-17), 42.8 (C-14), 41.6* (C-2′), 40.8 (C-8), 40.0 (C-22), 38.3 (C-1), 38.0 (C-13), 37.7 (C-4), 37.0 (C-10), 36.5* (C-4′), 35.5 (C-16), 34.2 (C-7), 31.9* (CH_2), 34.2* (CH_2), 29.7* (5x CH_2), 29.6* (3x CH_2), 29.8 (C-21), 29.3* (CH_2), (28.0 (C-23), 27.4 (C-15), 25.1 (C-12), 25.0* (CH_2), 23.7 (C-2), 22.6* (CH_2), 20.9 (C-11), 19.3 (C-30), 18.2 (C-6), 18.0 (C-28), 16.6 (C-25), 16.1 (C-24), 16.0 (C-26), 14.6 (C-27), 14.1* (C-18′). *****(acide gras)

EI-MS (70 eV) m/z (rel. Int.)= 708 (M^+, 65), 680 (70), 653 (5), 625 (4), 623 (4), 599 (7), 598 (7), 569 (6), 469 (12), 468 (31), 411 (58), 410 (100), 409 (62), 394 (24), 304 (24), 303 (25), 230 (26), 220 (33), 219 (94), 217 (32), 216 832), 191 (93), 190 (86), 189 (97), 175 (33) 147 (32), 136 (45), 135 (64), 123 (47), 121 (52), 95 (59), 91 (38), 83 (28), 82 (24), 81 (40), 69 (46), 68 (20).

3.4. Compound.4

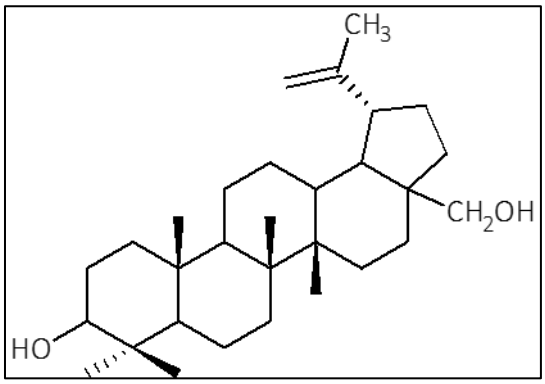
[3β,28-Dihydroxylup-20(29)-ène]

Gross Formula : $C_{30}H_{50}O_2$

^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm)= 4.67 (1H, br s, H-29a), 4.57 (1H, br s, H-29b), 3.80 (1H, d, J= 10.7 Hz, H-28a), 3.33 (1H, d, J= 10.7 Hz, H-28b), 3.19 (1H; dd; J=10.9, 5.3 Hz, H- 3), 2.38 (1H, qd, H-19), 1.91 (1H, m, H-21a), 1.90 (1H, m, H-22a), 1.87 (1H, m, H-16a), 1.85 (1H, m, H-7a), 1.70 (1H, m, H-15a),1.68 (3H, s, H3-30), 1.64 (2H, m, H-1a,12a), 1.61 (2H, m, H-2a,13), 1.58 (1H, H-18), 1.56 (1H, m, H-2b), 1.54 (1H, m, H-6a), 1.42 (1H, m, H-11a), 1.39 (1H, m, H-6b), 1.25 (2H, m, H-9,21b), 1.21 (2H, m, H-11b,21b), 1.07 (2H, m, H- 12b,15b), 1.06 (2H, m, H-7b,16b), 1.02 (3H, s, H3-26), 0.98 (3H, s, H3-27), 0.96 (3H, s, H3- 23), 0.89 (1H, m, H-1b), 0.82 (3H, s, H3-25), 0.76 (3H, s, H3-24), 0.68 (1H, H-5).

^{13}C-NMR (100.5 MHz, $CDCl_3$): δ(ppm)= 150.4 (C-20), 109.6 (C-29), 78.9 (C-3), 60.5 (C- 28), 55.2 (C-5), 50.3 (C-9), 48.7 (C-18), 47.7 (C-17,19), 42.6 (C-14), 40.8 (C-8), 38.8 (C-4), 38.7 (C-1), 37.3 (C-13), 37.1 (C-10), 34.2 (C-16), 33.9 (C-7), 29.7 (C-21), 29.1 (C-22), 27.9 (C-23), 27.3 (C-2), 27.0 (C-15), 25.2 (C-12), 20.8 (C-11), 19.0 (C-30), 18.3 (C-6), 16.1 (C-25), 15.9 (C-26), 15.3 (C-24), 14.7 (C-27).

EI-MS (70 eV) m/z (rel. Int.)= 442 (M^+, 44), 424 (16), 413 (26), 412 (100), 298 (12), 236 (14), 235 (18), 208 (18), 203 (26), 191 (21), 190 (14), 189 (22), 187 (16), 135 (23), 121 (16), 119 (15), 109 (15), 95 (19), 81 (18), 69 (11).



Compound.4: [3β,30-Dihydroxylup-20(29)-ène]

Gross Formula: $C_{30}H_{50}O_2$

PF: 236-237 °C.

Rf= 0.49 (Chloroform/Methanol 95/5)

Molecular ion at m/z: 442 [M^+, 45]

4. Conclusion

Ethnobotanical surveys conducted in western Madagascar have led to the selection of a plant known by the vernacular name of Voatsokopika and called *Carissa sessiliflora Brongn*. ex Pichon (Apocynaceae). This plant is held in high esteem in this region due to its therapeutic properties.

The local population utilizes this substance for the treatment of infections and fever. The crude hydro-ethanolic extract from the bark of this plant's stems has been shown to possess antimalarial effects in both in vivo and *in vitro* models, while all fractions derived from this extract have been found to be inactive. The application of fractionation to the cyclohexane extract, followed by a series of chromatographic analyses, has enabled the isolation of four pure products. The chemical structure of these compounds was established using spectroscopic analyses by nuclear magnetic resonance (NMR) (1D and 2D) and mass spectrometry (electrospray ionization (ESI)-MS, 70 eV in positive mode), and all four compounds belong to the pentacyclic triterpene family. The following compounds were identified: 3β-hydroxylup-20(29)-ene (1); 3β,30-dihydroxylup-20(29)-ene (2); lupeol 3-dihydroxyoctadecanoate (3); and 3β,28-dihydroxylup-20(29)-ene (4). The findings indicate that the antimalarial activity exhibited by *Carissa sessiliflora Brongn.* ex Pichon stem bark extract is attributable to the synergistic effect of multiple molecules present within this extract. These results also serve to reinforce the ethnobotanical data on this plant and complete the chemotaxonomy of *Carissa sessiliflora Brongn.*

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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