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## **Antiplasmodial and Cytotoxic Activity of Lanostane Type Triterpenoids isolated from *Leplaea mayombensis***

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## Abstract

Leplaeric acid **5**, leplazarin **6a** and 21-epileplazarin **6b**, three new 3,4-*seco*-lanostane type triterpenes have been isolated from the stem bark of *Leplaea mayombensis* (Pellegr.) Staner along with fourteen known compounds from the fruits and roots. Leplaeric acid E, leplazarin and 21-epileplazarin, 15- $\alpha$ -hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid, mayomlactones A and B, lanosta-7,24-dien-3-one, leplaeric acid A, B and C were screened *in vitro* for antiplasmodial activity against chloroquine-sensitive (*Pf3D7*) and chloroquine-resistant (*PfINDO*) strains of *Plasmodium falciparum* and for cytotoxicity against CAL-27, CaCo2, Skov-3, and HepG2 cells line. Three compounds including 15- $\alpha$ -hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid (IC<sub>50</sub> 5.65-7.09  $\mu$ M), lanosta-7,24-dien-3-one (IC<sub>50</sub> 7.18-9.07  $\mu$ M), and leplaeric acid C (IC<sub>50</sub> 7.59-8.47  $\mu$ M) were the most active against both strains of *P. falciparum*. All the compounds exhibited cytotoxicity against the three-cell lines with IC<sub>50</sub> ranging from 12.30 to 181.88  $\mu$ M. These results confirm the usage of the medicinal plant *L. mayombensis* for the management of malaria and suggest that further lead optimization studies on potent compounds identified from this study could lead to the identification of potential of lead molecules as scaffold for new antimalarial drug discovery.

**Keywords:** *Leplaea mayombensis*, Lanostanes, Triterpenes, Antiplasmodial activity, Cytotoxicity.

## 1. Introduction

Despite the emergence of new infectious diseases such as the recent outbreak of the COVID-19 (WHO,2020) malaria still remains a major public health concern worldwide, with the African region being disproportionately affected. Among the about 1.5 million lives claimed each year by malaria, 96% of deaths occurred in Africa, with children under 5 years of age accounting for about 80% of all deaths (WHO, 2021) In recent years, antimalarial drug resistance has emerged as a threat to global malaria control and has significantly challenged the malaria elimination agenda (Menard and Dondorp, 2017). Therefore, there is a need to develop new and more efficient antimalarials with novel mode of actions to combat resistant pathogens and reduce the burden of this infectious disease (Ariey *et al.*, 2014).

Historically, medicinal plants have provided medicines to fight against malaria as exemplified by quinine from *Cinchona* spp. and artemisinin from *Artemisia* spp. Hence, investigating plants currently used in traditional medicine against this disease can provide future drugs (Koumba *et*

*al.*, 1998; Koenen, and De Wilde, 2012; Atanasov *et al.*, 2015). The present study aimed to investigate the antiplasmodial potential of metabolites from *Leplaea mayombensis* (Pellegr.) Staner. (Meliaceae) from Cameroon. Several parts of this medicinal plant is currently used by local populations of the Central region of Cameroon for the management of malaria, syphilis, fever, and other infectious diseases (Kingston and Newman, 2012; Kinghorn, 2015; Farhaa and Brown, 2016). In our previous investigations several metabolites from the roots of *L. mayombensis* including 3,4-*seco*-lanostane type triterpenoids, steroids and flavonoids were found to exhibit cytotoxic activity (Sidjui *et al.*, 2017).

## 2. Results and Discussion

### 2.1. Isolation and structure elucidation

Leplaeric acid E (**5**) was obtained as a white microcrystalline powder. Its molecular formula was determined as C<sub>32</sub>H<sub>50</sub>O<sub>4</sub> on the basis of HR-ESI-MS m/z 521.3614 [M + Na]<sup>+</sup> (calcd, C<sub>32</sub>H<sub>50</sub>O<sub>4</sub>: 521.3601), indicating eight degrees of unsaturation. The IR spectrum showed bands at 1731 cm<sup>-1</sup> for the carboxylic carbonyl as well as the band at 1633 cm<sup>-1</sup> for olefinic functionality. The <sup>1</sup>H and <sup>13</sup>C NMR spectra showed close similarity to those of leplearic acid A previously isolated from the roots of the same plant (Sidjui *et al.*, 2017), implying that both compounds have the same basic C-30 skeleton (Table 1) with six methyl carbons ( $\delta_C$  27.6, 25.8, 22.7, 22.0, 17.8, 16.0), nine methylene carbons ( $\delta_C$  33.8, 32.5, 31.9, 30.4, 30.3, 29.8, 28.4, 26.2, 18.1), one methyldene group (=CH<sub>2</sub>,  $\delta_C$  114.0), six methine carbons including four sp<sup>3</sup> methine ( $\delta_C$  50.0, 49.5, 47.1, 40.7), two sp<sup>2</sup> methine ( $\delta_C$  123.7, 118.5), two carbonyls ( $\delta_C$  180.1, 174.2), three fully substituted olefinic carbons ( $\delta_C$  147.5, 146.1, 132.5), and three quaternary carbons ( $\delta_C$  51.4, 43.4, 37.0). The main difference between the two compounds was observed in the <sup>1</sup>H-NMR spectra which showed the presence of a carboethoxy moiety at C-3: the signal of oxymethylene protons being observed as quartet at  $\delta_H$  4.10 (2H, *J* = 7.1 Hz, H-31) while the methyl group resonated as triplet at  $\delta_H$  1.25 (3H, *J* = 7.1 Hz, H-32). The corresponding carbons were observed at  $\delta_C$  60.4 (C-31) and 14.4 (C-32), respectively. Moreover, the signal of C-3 was observed upfield resonance at  $\delta_C$  174.2 compared to that of leplearic acid A at  $\delta_C$  181.3 revealing an ester function. The presence and the position of the ethoxyl group at C-3, was confirmed by HMBC spectra which showed <sup>2,3</sup>J<sub>HC</sub> crosspeak correlation between signals at  $\delta_H$  4.10 (H-31),  $\delta_H$  2.36 (H-2a) and  $\delta_H$  2.18 (H-2b) with signal at  $\delta_C$  174.2 (C-3) (Figure 2). Basic on this evidence using COSY and HMBC experiments (Figure 2), the structure of leplaeric acid E (**5**) was elucidated as (5*R*,9*S*,10*R*,13*R*,14*R*,17*R*,20*R*)-3,4-*seco*-lanostan-4 (28),7,24-triene-21-oic acid 3-ethyl ester. It may have been an artefact arising from leplearic acid A, but this probability was ruled out by extraction of the same plant material with EtOAc and subsequent

HPLC of the EtOAc extract which showed the presence of compound **5** by comparison of retention time. Thus, compound **5** was concluded to be a genuine natural product.

Compounds **6a** and **6b** were each obtained as a white microcrystalline powder and gave brisk effervescence with dilute sodium bicarbonate solution indicating the presence of a free carboxylic moiety. The molecular formula was determined as  $C_{30}H_{46}O_4$  basis on HR-ESI-MS which showed a pseudomolecular  $[M + Na]^+$  peak at  $m/z$  493.3277 indicating eight degrees of unsaturation. The IR spectrum showed bands at  $3599\text{ cm}^{-1}$  for the hydroxyl group,  $1704\text{ cm}^{-1}$  for the carboxylic carbonyl and  $1654\text{ cm}^{-1}$  for the olefinic functionality. The NMR spectra revealed that the compound is in fact an epimeric mixture exhibiting many close pairs of signals (Table 1). From the integrals of signal in the  $^1\text{H-NMR}$  spectrum showed a mixture of **6a/6b** at a ratio of 60/40 (signal  $\text{CH}_3$ -18, (**S12**)). The NMR signals of the cyclic skeleton of both isomers showed close similarity to those of leplacric acid **E** (**5**). This was further confirmed by similar correlations in 2D-NMR experiments (COSY, HSQC, HMBC and NOESY) (Figure 2). The only notable difference was in the signals of the side chains C-20 to C-27. In case of both epimers, the NMR signals showed close resemblance to those of 21,23-epoxy-7 $\alpha$ -21-dihydroxyapotirucalla-14,24-dien-3-one (Kamperdick *et al.*, 2003). The hemiacetal function of the side chain was also indicated by signals of C-21 ( $\delta_C$  101.6 / 97.5 and  $\delta_H$  5.31 / 5.29). However, no signals of acetyl or methoxyl group were observed, leaving the hemiacetal hydroxyl group unsubstituted and prone to epimerization at C-21, which explains the existence of both the epimers. NOESY spectra cannot determinate which epimer, but a computer modelling of the two C-21 epimer (Figure 3) may explain the presence or absence of  $^3J_{HC}$  crosspeak in the HMBC (with  $J_{HC}$  long range = 8 Hz optimisation) spectra between H-21 and C-22 (S.21); the C-21 in *R* configuration epimer showed a dihedral angle of  $85^\circ$  between H-21 and C-22, and the C-21 in *S* configuration epimer showed a dihedral angle of  $149^\circ$  between H-21 and C-22 (fig. 4). Within the Karplus equation (Breitmaier, E. and W. Voelter, 1990.), the values of the synclinal carbon-proton (dihedral angle close to  $90^\circ$ ) couplings constants are generally smallest ( $<2$  Hz). Hence, epimer *R* may attribute to compound **6a** without  $^3J_{HC}$  crosspeak in the HMBC spectra between H-21 and C-22. Conversely, the values of the antiperiplanar carbon-proton (dihedral angle close of  $0^\circ$  and  $180^\circ$ ) coupling constants are generally largest (around 8 Hz). Hence, *S* epimer may attribute to compound **6b** with the presence of  $^3J_{HC}$  crosspeak in the HMBC spectra between H-21 and C-22. Compounds **6a** and **6b** were thus defined as new seco-lanostane-type triterpenoids and named leplazarin **6a** and 21-epileplazarin **6b**, respectively.

In addition, friedelan-3 $\beta$ -ol **1** (Sidjui *et al.*, 2015) docosanoic acid **2** (Gaydou *et al.*, 1993), friedelanone **3** (Mahato and Kundu, 1994), stigmasterol **4** (Sidjui *et al.*, 2015), 15- $\alpha$ -hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid **7** (Kamperdick *et al.*, 1997), 5- $\alpha$ -hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid 3-methyl ester **8** (Xia *et al.*, 2015),  $\beta$ -sitosterol **9** (Sidjui *et al.*, 2015), 3-O- $\beta$ -D-glucopyranosyl  $\beta$ -sitosterol **10** (Sidjui *et al.*, 2015) and 15 $\alpha$ -acetoxy-3-hydroxytrametenolic acid **11** (Xia *et al.*, 2015), Mayomlactones A **12** and B **13** (Sidjui *et al.*, 2019), lanosta-7,24-dien-3-one **14** (Sidjui *et al.*, 2019), leplaeric acid A **15**, B **16** and C **17** (Sidjui *et al.*, 2017) are known compounds among which **12** and **13** were previously isolated from fruits and compounds **14-17** from roots of *L. mayombensis*.

## 2.2. Biological assay

### 2.2.1. Antiplasmodial assay

Crude extracts and compounds were evaluated *in vitro* for their antiplasmodial potential against the chloroquine-sensitive (*Pf3D7*) and chloroquine-resistant (*PfINDO*) strains of *P. falciparum*. Fruits (IC<sub>50</sub> 7.39  $\mu$ g/mL), roots (IC<sub>50</sub> 11.4  $\mu$ g/mL) and stem barks (IC<sub>50</sub> 7.4  $\mu$ g/mL) extracts exhibited good potency against *Pf3D7*, while only extract from fruit inhibited *PfINDO* (IC<sub>50</sub> 9.05  $\mu$ g/mL). The antiplasmodial potency of extracts from different organs of *L. mayombensis* not only supports the traditional usage against malaria, but also suggests that further phytochemical investigation could reveal potential active ingredients. Therefore, extracts were submitted to fractionation and purified compounds were screened for antiplasmodial activity.

Overall, out of the 17 compounds, only 10 (were in sufficient amount to be tested) were screened and the results are summarised in Table 2. The compounds exhibited varying degrees of activity with IC<sub>50</sub> values ranging from 5.65-27.81  $\mu$ M and 7.09-38.25  $\mu$ M against *Pf3D7* and *PfINDO*, respectively. Out of the 10 compounds, three including 15- $\alpha$ -hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid **7** (IC<sub>50</sub> 5.65-7.09  $\mu$ M), lanosta-7,24-dien-3-one **14** (IC<sub>50</sub> 7.09-9.07  $\mu$ M) and leplaeric acid C **17** (IC<sub>50</sub> 7.59-8.47  $\mu$ M) exhibited very good activity with IC<sub>50</sub> below 10  $\mu$ M. These three compounds represent good potential candidates for *in vivo* efficacy and pharmacomodulation studies. The results are also in agreement with existing reports on the potential antiplasmodial activity of lanostanes (Katja *et al.*, 2016).

### 2.2.2. Cytotoxicity assay

The 17 compounds were further submitted to antiproliferative activity *in vitro* against three cell lines (Table 3). All tested compounds exhibit certain degree of inhibition with recorded IC<sub>50</sub> values ranging from 12.30 to 181.88 μM. Out of the 17, only compound **17** was active against CAL cell line (IC<sub>50</sub> 12.30 ± 0.08 μM), while against CaCo2 cell line, compounds **5**, **7**, **8**, and **13-16** were moderately active with IC<sub>50</sub> values ranging 14.07-39.42 μM. Compounds **5**, **8**, **13**, **15** and **17** exhibited activity against Skov-3 cell line (IC<sub>50</sub> 16.78-63.30 μM). Some lanostane-type triterpenoids isolated from plants of the Meliaceae family were also reported for their cytotoxicity (Wang *et al.*, 2008; Katja *et al.*, 2016; Hongthong *et al.* 2016). Overall, none of the 17 compounds was more potent than doxorubicin (IC<sub>50</sub> 1.54-4.96 μM) used as the positive control against the three cell lines.

## 3. Experimental

### 3.1. General experimental procedures.

The IR spectra were recorded on a Bruker Tensor 27 Spectrometer. The NMR spectra were recorded on a Varian Inova-500 NMR spectrometer at 500 MHz (<sup>1</sup>H), Bruker Avance II<sup>+</sup> 600 spectrometer at 600 MHz (<sup>1</sup>H) equipped with a 5 mm TCI Cryoprobe in 2.0 mm o.d. capillary tube at 300K. The chemical shifts are reported in δ values in ppm relative to CDCl<sub>3</sub> (δ<sub>1H</sub> 7.26 ppm, δ<sub>13C</sub> 77.16 ppm), coupling constants (*J*) are reported in hertz (Hz), unless indicated otherwise. The HR-ESI-MS were obtained using a micrOTOF (Bruker) mass spectrometer. Column chromatography was performed over silica gel (230–400 mesh), and vacuum liquid chromatography was performed over silica gel (70–230 mesh). Thin-layer chromatography (TLC) was carried out on pre-coated silica gel 60 F<sub>254</sub> plates (Merck), and the TLC spots were viewed at 254 nm and visualized by heating the plates at 80 °C for 10 min after spraying with 50 % aqueous sulfuric acid.

**Molecular mechanics calculations:** The implementation of the MMFF94 force field (Halgren, T.A. (1996)) in the Avogadro software: an open-source molecular builder and visualization tool. Version 1.2.0 (<http://avogadro.cc/>) was used to calculate the molecular models.

### 3.2. Plant material

Fruits, roots and stembarks of *L. mayombensis* were harvested in Yaoundé-Cameroon, (October 2014) and identified by Mr Victor Nana at the Cameroon National Herbarium (HNC), where a voucher specimen has been deposited (46220/HNC).

### 3.3. Extraction and isolation

The air-dried and powdered fruits (1.5 kg) (LMF), roots (1.5 kg) (LMR) and stem barks (2.5 kg) (LMS) of *L. mayombensis* were extracted with MeOH-DCM (1:1, v/v) mixtures by maceration at room temperature for 72 h. The filtrates were dried under vacuum to yield LMF (180.5 g), LMR (240.2 g) and LMS (250.5 g) extracts, respectively.

The LMS extract was subjected to vacuum liquid chromatography over silica gel eluting with hexane, hexane/ EtOAc (7.5:2.5), hexane/ EtOAc (7:3), hexane/ EtOAc (1:1), EtOAc, EtOAc / MeOH (9:1) and MeOH to afford seven fractions labelled LMS1 (19.20 g), LMS2 (25.30 g), LMS3 (20.15 g), LMS4 (44.28 g), LMS5 (62.08 g), LMS6 (10.25 g) and LMS7 (60.20 g), respectively. Fractions LMS1, LMS2, LMS3, LMS4 and LMS5 were pooled together on the basis on their TLC profile into two pools LMSa (62.65 g) and LMSb (106.36 g). Pool LMSa was subjected to vacuum liquid column chromatography over silica gel (70–230 mesh) and eluted with n-hexane/EtOAc (1:0-8:2), to afford friedelan-3 $\beta$ -ol **1** (3.7 mg) and docosanoic acid **2** (4 mg). Elution with n-hexane/EtOAc (8:2-6:4) provided friedelanone **3** (4 mg). Elution with n-hexane/EtOAc (6:4-5:5) provided stigmasterol **4** (6 mg), leplaeric acid E **5** (10 mg) and a binary mixture of compounds (10 mg). The latter was separated by column chromatography using CHCl<sub>3</sub>/acetone (1:0-7:2), collecting small sous fractions to obtain a mixture of leplazarin **6a** (3 mg) and 21-epileplazarin **6b** (3.5 mg). The latter was separated by column chromatography using CHCl<sub>3</sub>/acetone (1:0-7:2). The pooled LMSb (106.36 g) was subjected to vacuum liquid column chromatography silica gel (70–230 mesh) and eluted with CHCl<sub>3</sub>/MeOH (1:0-7:3) to provide 15- $\alpha$ -hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid **7** (4 mg), 5- $\alpha$ -hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid 3-methyl ester **8** (3.5 mg),  $\beta$ -sitosterol **9** (5.7 mg) and 3-*O*- $\beta$ -D-glucoopyranosyl  $\beta$ -sitosterol **10** (4.7 mg). Increasing the polarity with EtOAc/MeOH (5:5-0:1) led to the isolation of 15 $\alpha$ -acetoxyl-3-hydroxytrametenolic acid **11** (4 mg) (Figure 1). Compounds **12-13** were obtained from fruits while **14-17** were isolated from roots of *L. mayombensis* by following a previously reported protocol (Sidjui *et al.*, 2017; Zia-UI-Haq *et al.*, 2013).



### 3.3.1 leplaeric acid E 5

White, microcrystalline powder, mp: 198-199 °C; IR  $\nu_{\max}$  1704, 1654  $\text{cm}^{-1}$ ;  $[\alpha]_{589}^{20} = +27,7^\circ$  (c 0.35,  $\text{CHCl}_3$ ), mp. 198-199°C;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; HR-ESI-MS  $m/z$  521.3614  $[\text{M} + \text{Na}]^+$  (calcd,  $\text{C}_{32}\text{H}_{50}\text{O}_4\text{Na}$ , 521.3601).

### 3.3.2 leplazarin 6a and 21-epileplazarin 6b

White, microcrystalline powder, mp: 125-126°C; IR  $\nu_{\max}$  3599, 1704, 1654  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; HR-ESI-MS:  $m/z$  493.3277  $[\text{M} + \text{Na}]^+$  (calcd,  $\text{C}_{30}\text{H}_{46}\text{O}_4\text{Na}$  493.3288).

## 3.4. Antiplasmodial assay

Chloroquine-sensitive *Pf*3D7 and chloroquine-resistant *Pf*INDO strains of *P. falciparum* were maintained in continuous culture in fresh  $\text{O}^{+\vee\text{e}}$  human erythrocytes suspended at 4% (v/v) haematocrit in complete RPMI 1640 medium [16.2 g/L RPMI 1640 (Sigma) containing 25 mM HEPES, 11.11 mM glucose, 0.2% sodium bicarbonate (Sigma), 0.5% Albumax I (Gibco), 45  $\mu\text{g}/\text{mL}$  hypoxanthine (Sigma) and 50  $\mu\text{g}/\text{mL}$  gentamicin (Gibco)] and incubated at 37 °C in an atmosphere of 5%  $\text{O}_2$ , 5%  $\text{CO}_2$ , and 90%  $\text{N}_2$ . The spent medium was replaced with fresh complete medium every day to propagate the culture. Giemsa-stained blood smears were examined microscopically under oil immersion to monitor cell cycle transitions and parasitaemia.

Prior to antiplasmodial activity testing synchronized ring stage parasites were obtained by 5% (w/v) sorbitol treatment as previously described (Lambros and Vanderberg, 1979). Using synchronized cultures over mixed-stage cultures can enable monitoring the effect of the test substances on all the three parasite stages (merozoite, trophozoite and schizont) throughout the 48 h life cycle of *P. falciparum* in culture. Moreover, starting the experiment with synchronized ring stage culture provides the distinct advantage of showing growth inhibitory effects without a rise in parasitemia during the ring-trophozoite-schizont transitions.

For drug activity testing, the SYBR green I based fluorescence assay was used as previously described (Smilkstein et al. 2004). The ability of SYBR green to emit strong fluorescence in the presence of DNA forms the basis for its use to assess cell proliferation. The absence of nucleus in human red blood cells where the malarial parasite proliferates allows the use of SYBR green for the specific monitoring of the parasite growth.

Ninety-six  $\mu\text{L}$  of sorbitol-synchronized ring stage parasites at 2% haematocrit and 1% parasitaemia under normal culture conditions were added to triplicate wells of the 96-well plates containing 4  $\mu\text{L}$  of each test substance and incubated for 48 h at 37°C in an atmosphere of 5%

O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% N<sub>2</sub>. CQ at 10 µM and 0.4% DMSO (v/v) were taken as positive (0% growth) and negative controls (100% growth) respectively. After 48 h of incubation, 100 µL of SYBR green I buffer [0.2 µl of 10,000 × SYBR Green I (Invitrogen) per mL of lysis buffer {Tris (20 mM; pH 7.5), EDTA (5 mM), saponin (0.008%; wt/vol), and Triton X-100 (0.08%; vol/vol)}] were added to each well, mixed gently and incubated in the dark at 37 °C for 1 hr. Fluorescence was subsequently measured using a Victor fluorescence multi-well plate reader (Perkin Elmer) with excitation and emission at 485 and 530 nm respectively. Fluorescence counts for CQ representing zero growth were subtracted from counts in each well. Dose–response curves were constructed by plotting mean fluorescence counts against the test substance concentrations and activity expressed as 50% inhibitory concentration (IC<sub>50</sub>) using the IC Estimator-version 1.2 software (Ninety-six µL of sorbitol-synchronized ring stage parasites at 2% haematocrit and 1% parasitaemia under normal culture conditions were added to triplicate wells of the 96-well plates containing 4 µL of each test substance and incubated for 48 h at 37°C in an atmosphere of 5% O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% N<sub>2</sub>. CQ at 10 µM and 0.4% DMSO (v/v) were taken as positive (0% growth) and negative controls (100% growth) respectively. After 48 h of incubation, 100 µL of SYBR green I buffer [0.2 µl of 10,000 × SYBR Green I (Invitrogen) per mL of lysis buffer {Tris (20 mM; pH 7.5), EDTA (5 mM), saponin (0.008%; wt/vol), and Triton X-100 (0.08%; vol/vol)}] were added to each well, mixed gently and incubated in the dark at 37 °C for 1 hr. Fluorescence was subsequently measured using a Victor fluorescence multi-well plate reader (Perkin Elmer) with excitation and emission at 485 and 530 nm respectively. Fluorescence counts for CQ representing zero growth were subtracted from counts in each well. Dose–response curves were constructed by plotting mean fluorescence counts against the test substance concentrations and activity expressed as 50% inhibitory concentration (IC<sub>50</sub>) using the IC Estimator-version 1.2 software (<http://www.antimalarial-icestimator.net/MethodIntro.htm>). Giemsa-stained smears of extract-treated parasite cultures were examined microscopically to validate the results from the fluorescence-based assay.

### **3.5. Cytotoxicity assay**

The cytotoxic effect of compounds was assessed using the MTT assay (Mosmann T, 1983), targeting the human epithelial colorectal adenocarcinoma cells (CaCo2), human ovarian caucasian adenocarcinoma cells (Skov-3), human caucasian hepatocytic carcinoma cells (HepG2), and squamous carcinoma cells (CAL-27) cultured in complete medium containing 13.5 g/l DMEM (Gibco, Waltham, MA USA), 10% foetal bovine serum (Gibco, Waltham, MA USA), 0.21% sodium bicarbonate (Sigma-Aldrich, New Delhi, India) and 50 µg/mL gentamicin

(Gibco, Waltham, MA USA). Essentially, cells line at  $5 \times 10^3$  cells/200  $\mu$ L/well were seeded into 96 – well flat-bottomed tissue culture plates (Corning, USA) in complete medium. 50  $\mu$ L of serially diluted compounds solutions ( $\leq 200 \mu\text{g/mL}$ ) were added after 24 h of seeding and cells plus test substance incubated for 48 h in a humidified atmosphere at 37°C and 5% CO<sub>2</sub>. DMSO (0.4 (v/v) was added as negative control (100% growth). Twenty microliters of a stock solution of MTT (5 mg/mL in 1X phosphate buffered saline) were added to each well, gently mixed and incubated for additional 4 h. After spinning the plate at 1,500 rpm for 5 min, the supernatant was carefully removed and 100  $\mu$ L of 100% DMSO (v/v) (stop agent) was added. Formazan formation was read on a microtiter plate reader (Versa Max Microplate Reader, Molecular Devices, USA) at 570 nm. The 50% cytotoxic concentrations (CC<sub>50</sub>) of compounds were determined by analysis of dose – response curves. Selectivity Indices (Mammalian cell CC<sub>50</sub>/*P.falciparum* IC<sub>50</sub>) were calculated for each compound.

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### **Competing financial interest**

The authors declare no conflict of interest.

### **Appendix A. Supporting information**

NMR raw data (<sup>1</sup>H, <sup>13</sup>C, gCOSY, gNOESY, gHSQC, gHMBC) of compound **5** and **6a/6b** are made freely available at <https://doi.org/10.5281/zenodo.6256653>. Supplementary data associated with this article can be found in the online version at doi:10.1016/j.phytol.2022.06.010.

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## Legends

### Figures

**Figure 1:** Compounds **5**, **6a** and **6b**

**Figure 2:** Relevant  $^1\text{H}$ - $^1\text{H}$  COSY and  $^{2,3}\text{J}_{\text{HC}}$  HMBC correlations of **5**, **6a** and **6b**

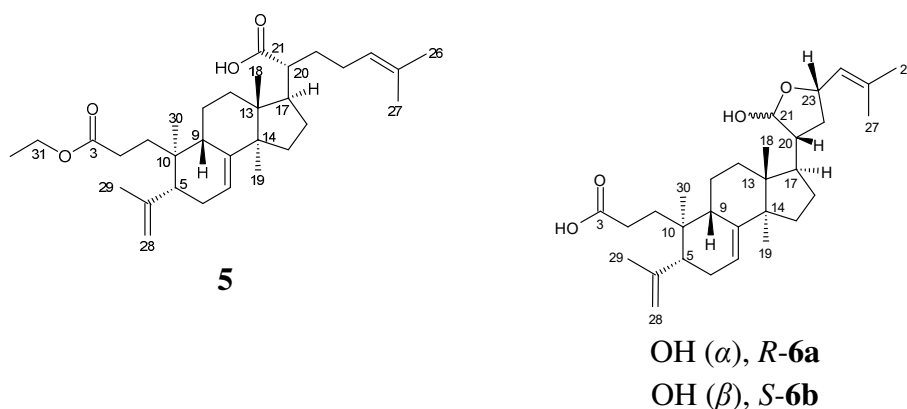
**Figure 3:** Computer-generated model, using MMFF94 force field calculation and Newman projections for C-21, C-20 bonds in (a) (R)-leplazarin **6a** and (b) (S)-21-epileplazarin **6b**.

### Tables

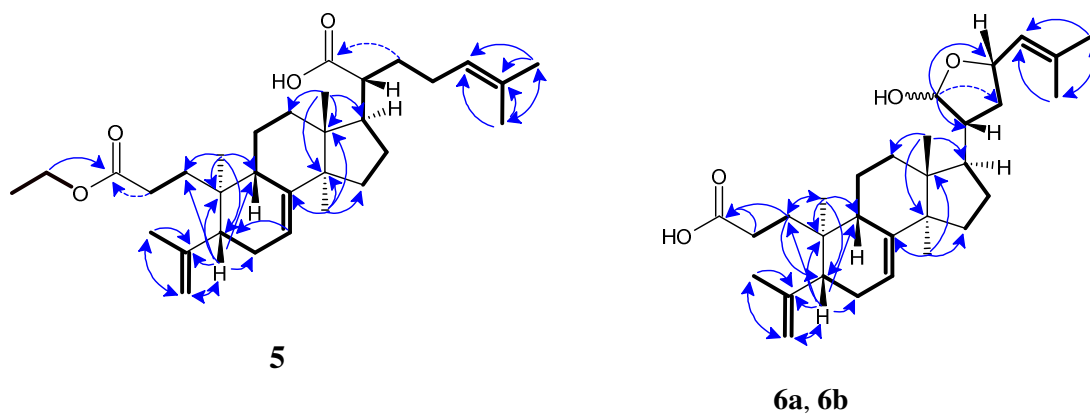
**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for **5** (500 and 125 MHz) and **6a/6b** (600 and 150 MHz) in  $\text{CDCl}_3$  at 300K

**Table 2:** *In vitro* antiplasmodial activity of crude extracts and pure compounds isolated from *L. mayombensis* on both chloroquine-sensitive (*Pf3D7*) and chloroquine-resistant (*PfINDO*) strains of *Plasmodium falciparum*, antiproliferative activity on human caucasian hepatocytic carcinoma cells and selectivity indexes.

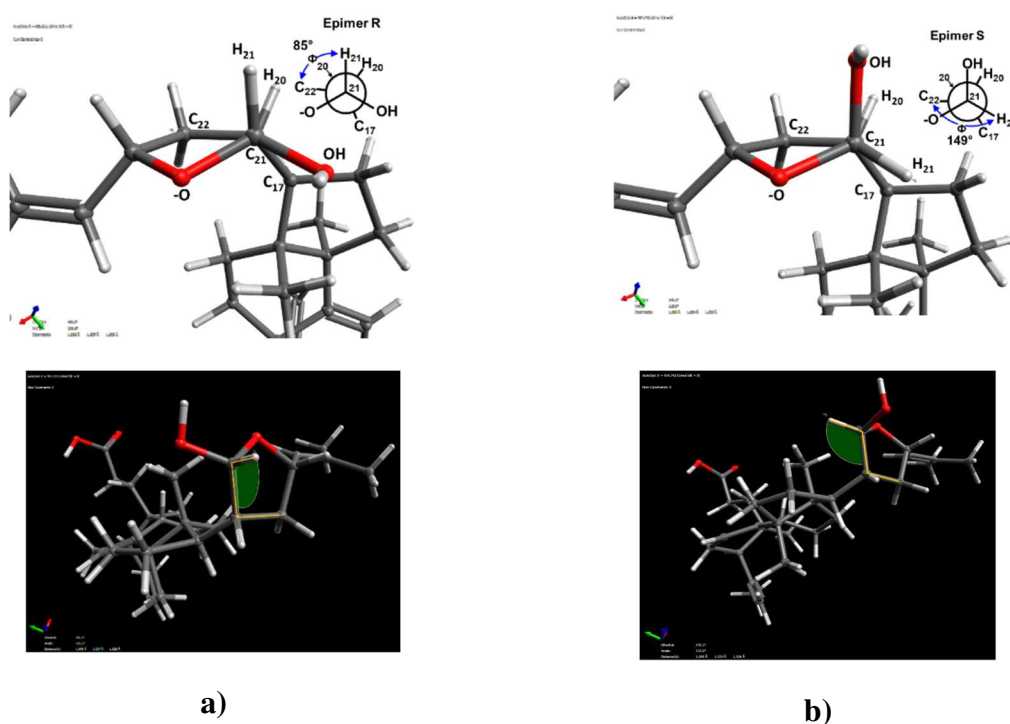
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**Table 1.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR data for **5** (500 and 125 MHz) and **6a/6b** (600 and 150 MHz) in  $\text{CDCl}_3$  at 300K

atom	<b>5</b>		<b>6a</b>		<b>6b</b>	
	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$
1	1.64 (1H, m)	31.9	1.76 (1H, m)	31.7	1.76 (1H, m)	31.7
	1.55 (1H, m)		1.64 (1H, m)		1.64 (1H, m)	
2	2.36 (1H, m)	28.4	2.43 (1H, m)	27.9	2.43 (1H, m)	27.9
	2.18 (1H, m)		2.26 (1H, m)		2.26 (1H, m)	
3	-	174.2	-	178.6	-	178.6
4	-	147.5	-	147.5	-	147.5
5	2.42 (1H, dd, 11.9, 5.8)	49.5	2.41 (1H, dd, 11.8, 4.3)	49.6	2.41 (1H, dd, 11.8, 4.3)	49.8
6	2.18 (1H, m)	30.3	2.20 (1H, m)	30.3	2.20 (1H, m)	30.3
	2.03 (1H, m)		2.03 (1H, m)		2.03 (1H, m)	
7	5.25 (1H, dd 6.6, 3.2)	118.5	5.25 (1H, m)	118.3	5.26 (1H, m)	118.2
8	-	146.1	-	146.2	-	146.3
9	2.44 (1H, m)	40.7	2.50 (1H, m)	40.8	2.50 (1H, m)	40.8
10	-	37.0	-	36.9	-	36.9
11	1.48 (2H, m)	18.1	1.56 (1H, m)	18.0	1.56 (1H, m)	18.0
			1.52 (1H, m)		1.52 (1H, m)	
12	1.75 (1H, m)	30.4	1.76 (1H, m)	31.7	2.04 (1H, m)	31.5
	1.48 (1H, m)		1.64 (1H, m)		1.64 (1H, m)	
13	-	43.4	-	43.6	-	43.6
14	-	51.4	-	51.4	-	51.2
15	1.50 (2H, m)	33.8	1.55 (1H, m)	34.1	1.55 (1H, m)	34.5
			1.49 (1H, m)		1.49 (1H, m)	
16	1.25 (2H, m)	29.8	1.89 (1H, m)	27.5	1.89 (1H, m)	27.7
			1.32 (1H, m)		1.32 (1H, m)	
17	2.04 (1H, m)	50.0	1.79 (1H, m)	50.7	2.07 (1H, m)	45.2
18	0.93 (3H, s)	22.0	0.94 (3H, s)	22.9	0.88 (3H, s)	23.5
19	1.00 (3H, s)	27.6	1.00 (3H, s)	27.5	1.02 (3H, s)	27.7
20	2.32 (1H, m)	47.1	2.22 (1H, m)	50.3	2.05 (1H, m)	47.7
21	-	180.1	5.31 (1H, d, 3.4)	101.6	5.29 (1H, d, 3.4)	97.5
22	1.58 (2H) m	32.5	2.12 (1H, ddd, 12.0, 7.2, 4.8)	39.8	2.00 (1H, m)	35.7
			1.28 (1H, m)		1.62 (1H, m)	
23	1.94 (2H, m)	26.2	4.82 (1H, ov)	74.2	4.72 (1H, td, 9.4, 6.0)	76.2
24	5.09 (1H, tspt, 7.1, 1.3)	123.7	5.14 (1H, dspt, 8.5, 1.4)	124.7	5.24 (1H, dspt, 8.5, 1.4)	127.9
25	-	132.5	-	137.3	-	136.0
26	1.68 (3H, brs)	25.8	1.73 (3H, brd, 1.4)	26.0	1.72 (3H, brd, 1.4)	26.0
27	1.58 (3H, brs)	17.8	1.71 (3H, brd, 1.4)	18.5	1.69 (3H, brd, 1.4)	18.1
28	4.84 (1H, brs)	114.0	4.84 (1H, brs)	114.2	4.84 (1H, brs)	114.2
	4.79 (1H, brs)		4.80 (1H, brs)		4.80 (1H, brs)	
29	1.78 (3H, brs)	22.7	1.78 (3H, brs)	22.4	1.78 (3H, brs)	22.4
30	0.82 (3H, s)	16.0	0.84 (3H, s)	16.1	0.84 (3H, s)	16.1
31	4.10 (2H, q, 7.1)	60.4	/	/	/	/
32	1.25 (3H, t, 7.1)	14.4	/	/	/	/

**Table 2:** *In vitro* antiplasmodial activity of crude extracts and pure compounds isolated from *L. mayombensis* on both chloroquine-sensitive (*Pf3D7*) and chloroquine-resistant (*PfINDO*) strains of *Plasmodium falciparum* and antiproliferative activity on human caucasian hepatocytic carcinoma cells and selectivity indexes

Crudes extracts	IC <sub>50</sub> (µg/mL) ± SD				
	Antiplasmodial		Cytotoxicity	Selectivity index	
	<i>Pf3D7</i>	<i>PfINDO</i>	HepG2	SI <sup>1</sup>	SI <sup>2</sup>
LMF	7.39 ± 0.09	9.05 ± 0.55		/	/
LMR	11.40 ± 0.82	>100		/	/
LMS	7.40 ± 0.82	>100		/	/
Compounds	IC <sub>50</sub> (µM) ± SD				
<b>5</b>	22.81 ± 0.23	35.77 ± 0.44	41.53 ± 0.46	1.82	1.16
<b>7</b>	5.65 ± 0.12	7.09 ± 0.14	43.32±2.61	7.72	6.11
<b>8</b>	14.76 ± 0.09	18.08 ± 0.68	61.35 ± 0.46	4.15	3.39
<b>11</b>	11.41 ± 0.59	8.72 ± 0.66	142.60 ± 0.64	12.49	16.35
<b>12</b>	9.92 ± 0.49	11.24 ± 0.66	75.90 ± 0.45	7.65	6.75
<b>13</b>	27.81 ± 0.43	38.25 ± 0.45	28.28 ± 0.46	1.01	0.73
<b>14</b>	9.07 ± 0.12	7.18 ± 0.14	63.01 ± 0.56	6.94	8.77
<b>15</b>	20.53 ± 0.78	17.96 ± 0.45	150.31 ± 0.46	7.32	8.36
<b>16</b>	16.00 ± 0.78	>20	49.13 ± 0.46	3.07	/
<b>17</b>	8.47 ± 0.12	7.59 ± 0.14	29.16 ± 0.46	3.46	3.84
Chloroquine	0.04 ± 0.02	0.40± 0.06	/	/	/

SI<sup>1</sup>: selectivity index (IC<sub>50</sub> Cell line/IC<sub>50</sub> *Pf3D7*), SI<sup>2</sup>: (IC<sub>50</sub> Cell line/IC<sub>50</sub> *INDO*), Chloroquine was used as the antiplasmodial drug compound of reference. LMF: DCM/MeOH crude extract of fruit, LMR: MeOH crude extract of root, LMS: DCM/MeOH crude extract of stem back. Data represent the mean ± SD of three independent experiments.

**Table 3:** *In vitro* cytotoxicity of pure compounds isolated from *L. mayombensis* against human cancer cells lines, CAL-27, CaCo2, and Skov-3

Compounds	IC <sub>50</sub> (μM) ± SD <sup>b</sup>		
	CAL-27 <sup>b</sup>	CaCo2 <sup>b</sup>	Skov-3 <sup>b</sup>
<b>5</b>	52.67 ± 0.80	23.95 ± 0.53	19.32 ± 0.30
<b>7</b>	111.85 ± 2.90	33.78 ± 1.47	160.38 ± 0.48
<b>8</b>	72.44 ± 0.80	14.07 ± 0.03	17.26 ± 0.30
<b>11</b>	78.25 ± 0.30	150.55 ± 0.33	171.35 ± 0.45
<b>12</b>	96.20 ± 0.80	82.17 ± 0.03	181.88 ± 0.39
<b>13</b>	70.79 ± 0.11	15.98 ± 0.53	16.78 ± 0.20
<b>14</b>	174.65 ± 4.43	39.42 ± 1.47	63.30 ± 0.56
<b>15</b>	98.33 ± 0.80	16.88 ± 0.53	18.34 ± 0.30
<b>16</b>	110.47 ± 1.46	33.21 ± 0.37	106.12 ± 1.56
<b>17</b>	12.30 ± 0.08	180.12 ± 5.07	44.94 ± 0.63
Doxorubicin <sup>a</sup>	4.96 ± 1.84	4.32 ± 1.04	1.54 ± 0.08

<sup>a</sup> Doxorubicin was used as a drug compound of reference for human cell toxicity

<sup>b</sup> N100 μg/mL, data represent the mean ± SD of three independent experiments.