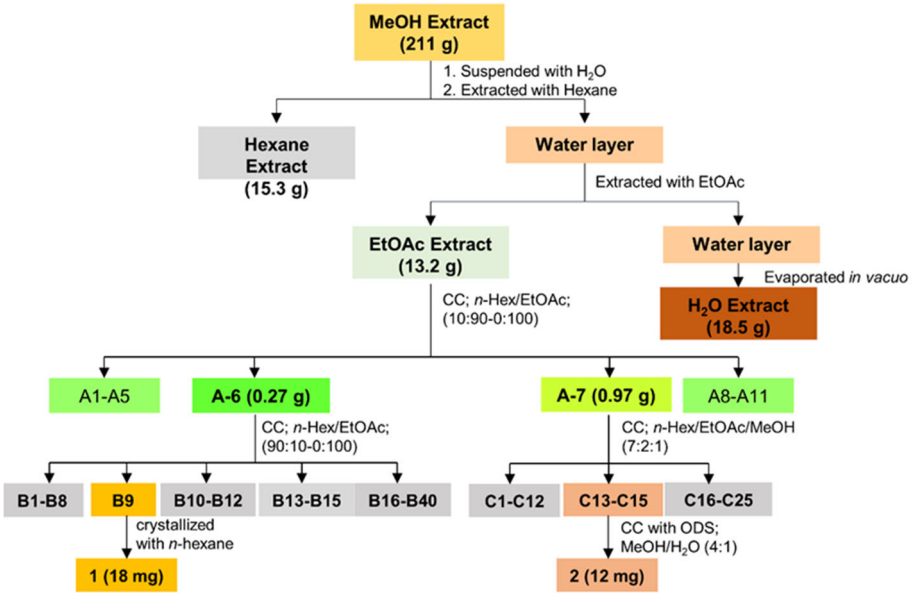
Supplementary Data

This supplementary data is a part of a paper entitled "Evaluation of the Antiplasmodial Properties of *Andrographis paniculata* (Burm.f.) and *Peperomia pellucida* (L.) Kunth".

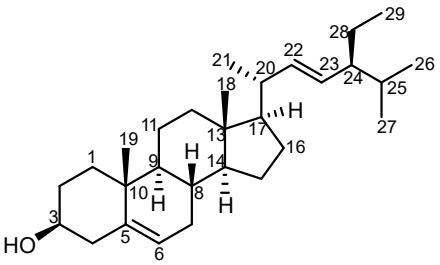
General information

Commercial grade solvents were used unless stated otherwise. Solvents for chromatography were distilled prior to use. Analytical thin layer chromatography (TLC) was performed on Kieselgel 60 F254 glass plates and Octa Desyl Silane (ODS) RP-18 (Merck). Spots were visualized by 10% sulfuric acid in ethanol. Silica column chromatography was performed on silica gel G60 (Merck) (230-400 mesh) and ODS RP-18.

Optical rotations were recorded with an ATAGO AP-300 polarimeter. InfraRed (IR) spectra were measured by using a PerkinElmer spectrum-100 FT-IR spectrometer in KBR. ^{1}H, ^{13}C and two-dimensional NMR spectra were measured with a JEOL JNM A-500 (500 MHz) spectrometer at 296 K. Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks (CD_3OD: δ 4.78 in ^{1}H-NMR). Chemical shifts (δ) are reported in parts per million (ppm) relative to the respective residual solvent peaks ($CDCl_3$: δ7.26 in ^{1}H and 77.20 in ^{13}C-NMR; CD_3OD: δ 4.78 in ^{1}H-NMR and 49.20 in ^{13}C-NMR). The following abbreviations are used to indicate peak multiplicities: s singlet; d doublet; dd doublet of doublets; t triplet; m multiplet. Coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were recorded using a Waters Xevo QTOF mass spectrometer.

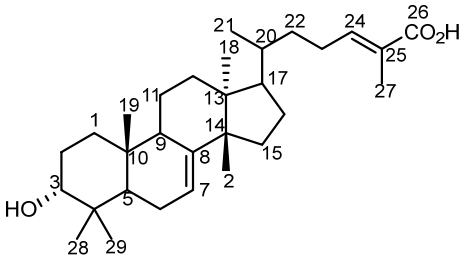


Scheme S1. Isolation procedures of compound **1**-**2**



3β-Hydroxy-24-ethyl-5,22-cholestadiene (1)

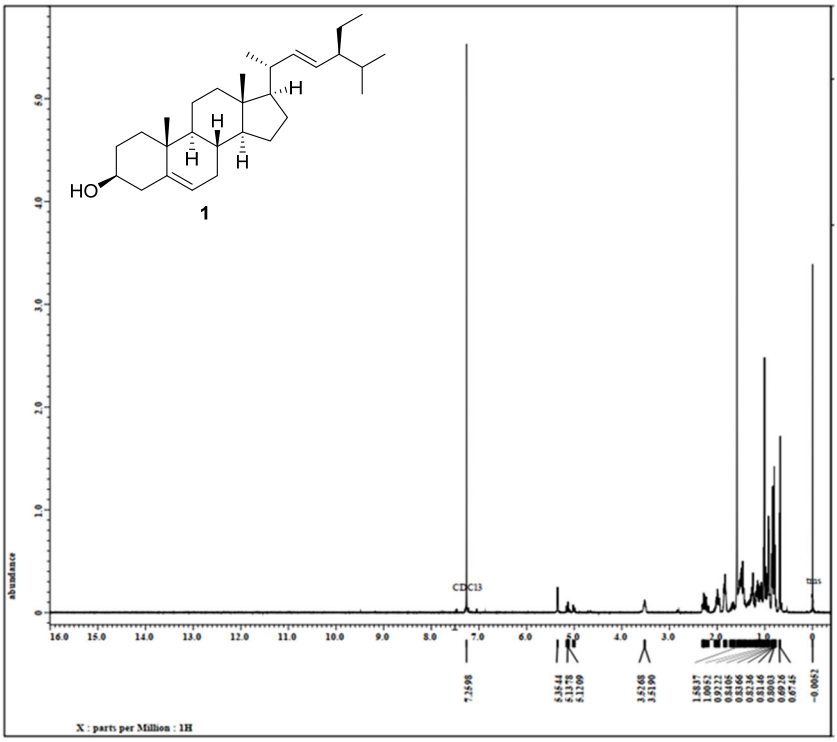
A white crystal, m.p 150–152 °C; $[\alpha]_D$ -38.6 (c 0.3, $CHCl_3$); IR (KBr)V_{max} 3401, 2861 1457 and 1039 cm^{-1}; ^{1}H-NMR (500 MHz, Chloroform-d). δ 1.08 (m, 1H, H-1), 1.84 (m, 1H, H-1'), 1.49 (m, 1H, H-2), 1.81 (m, 1H, H-2'), 3.52 (m, 1H, H-3), 2.28 dd, *J* = 2.0, 5.2 Hz, 1H, H-4), 2.30 (dd, *J* = 2.0, 5.2 Hz, 1H, H-4'), 5.35 (d, *J* = 5.2 Hz, 1H, H-6), 1.54 (m, 1H, H-7), 1.96 (m, 1H, H-7'), 1.46 (m, 1H, H-8), 0.94 (m, 1H, H-9), 1.46 (m, 1H, H-11), 1.49 (m, 1H, H-11'), 1.15 (m, 1H, H-12), 1.95 (m, 1H, H-12'), 1.03 (s, 1H, H-14), 1.07 (m, 1H, H-15), 1.56 (m, 1H, H-15'), 1.26 (m, 1H, H-16), 1.67 (m, 1H, H-16'), 1.13 (m, 1H, H-17), 0.67 (s, 3H, H-18), 1.00 (s, 3H, H-19), 2.02 (m, 1H, H-20), 0.92 (d, *J* = 9.5 Hz, 1H, H-21), 5.16 (dd, *J* = 8.5, 15.0 Hz, 1H, H-22), 5.00 (dd, *J* = 8.5, 15.0 Hz, 1H, H-23), 1.53 (m, 1H, H-24), 1.45 (m, 1H, H-25), 0.84 (d, *J* = 6.4 Hz, 3H, H-26), 0.82 (d, *J* = 6.1 Hz, 3H, H-27), 1.15 (t, *J* = 3.2 Hz, 1H, H-28), 0.80 (t, *J* = 6.0 Hz, 1H, H-29); ^{13}C-NMR (125 MHz, Chloroform-d). δ 37.4 (C-1), 31.8 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 21.3 (C-8), 50.3 (C-9), 36.7 (C-10), 21.3 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.4 (C-16), 56.1 (C-17), 12.1 (C-18), 19.5 (C-19), 40.7 (C-20), 21.2 (C-21), 138.5 (C-22), 129.5 (C-23), 51.4 (C-24), 31.8 (C-25), 21.3 (C-26), 19.1 (C-27), 25.6 (C-28), 12.2 (C-29); HR-TOFMS *m/z* 413.3748 $[M+H]^+$, (calculated for $C_{29}H_{48}O$, *m/z* 412.3704). In agreement with published data [1].



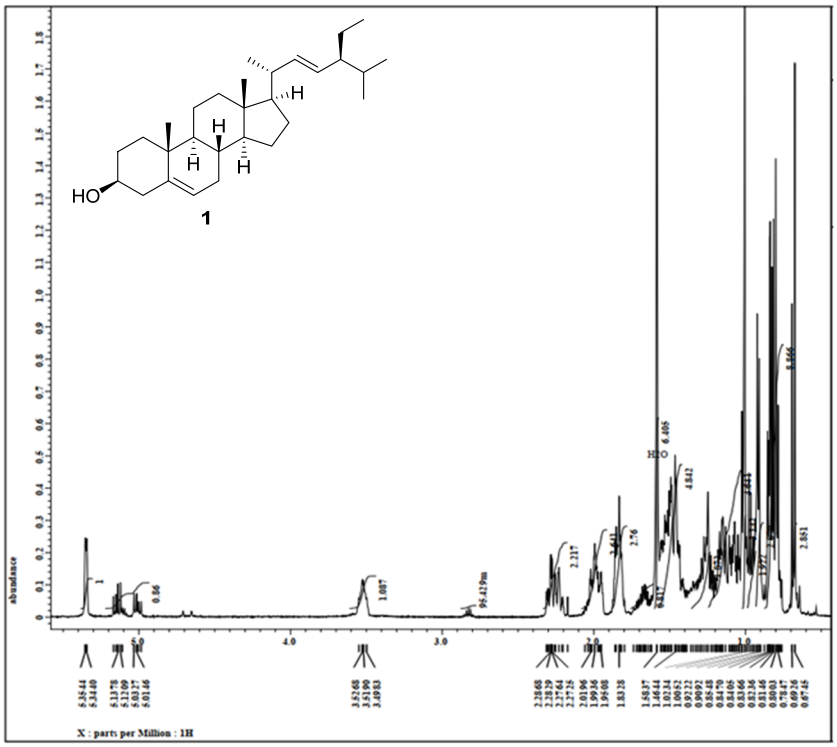
3β-hydroxy-9-lanosta-7,24*E*-dien-26-oic acid (2)

A white crystal, m.p 162–165 °C; $[\alpha]_D$ +15.6 (c 0.3, EtOH); IR (KBr)V_{max} 3241, 1701 1633 and 1120 cm^{-1}; ^{1}H-NMR (500 MHz, Methanol-d). δ 1.54 (m, 2H, H-1), 1.59 (m, 2H, H-2), 3.49 (dd, *J* = 4.0, 12.0 Hz, 1H, H-3), 1.45 (m, 1H, H-5), 1.85 (m, 2H, H-6), 5.26 (dd, *J* = 3.0, 6.5 Hz, 1H, H-7), 2.18 (m, 1H, H-9), 1.40 (m, 2H, H-11), 1.32 (m, 2H, H-12), 1.40 (m, 2H, H-15), 1.96 (m, 2H, H-16), 1.47 (m, 1H, H-17), 0.96 (s, 3H, H-18), 0.89 (s, 3H, H-19), 1.61 (s, 1H, H-20), 0.85 (d, *J* = 6.5 Hz, 3H, H-21), 1.51 (m, 2H, H-22), 2.02 (m, 2H, H-23), 5.98 (t, *J* = 7.8 Hz, 3H, H-24), 2.48 (s, 3H, H-27), 1.08 (s, 3H, H-28), 0.73 (s, 3H, H-29), 1.70 (s, 3H, H-30); ^{13}C-NMR (125 MHz, Methanol-d). δ 35.5 (C-1), 28.1 (C-2), 77.1 (C-3), 38.8 (C-4), 48.5 (C-5), 23.1 (C-6), 119.6 (C-7), 147.4 (C-8), 48.4 (C-9), 35.8 (C-10), 22.8 (C-11), 33.4 (C-12), 43.5 (C-13), 52.7 (C-14), 34.7 (C-15), 28.5 (C-16), 53.3 (C-17), 24.7 (C-18), 17.1 (C-19), 35.9 (C-20), 18.4 (C-21), 35.0 (C-22), 25.5 (C-23), 144.3 (C-24), 128.6 (C-25), 171.8 (C-26), 12.6 (C-27), 29.5 (C-28), 23.7 (C-29), 30.8 (C-30); HR-TOFMS *m/z* 455.3571 $[M-H]^-$, (calculated for $C_{30}H_{48}O_3$, *m/z* 456.3503). In agreement with published data [2].

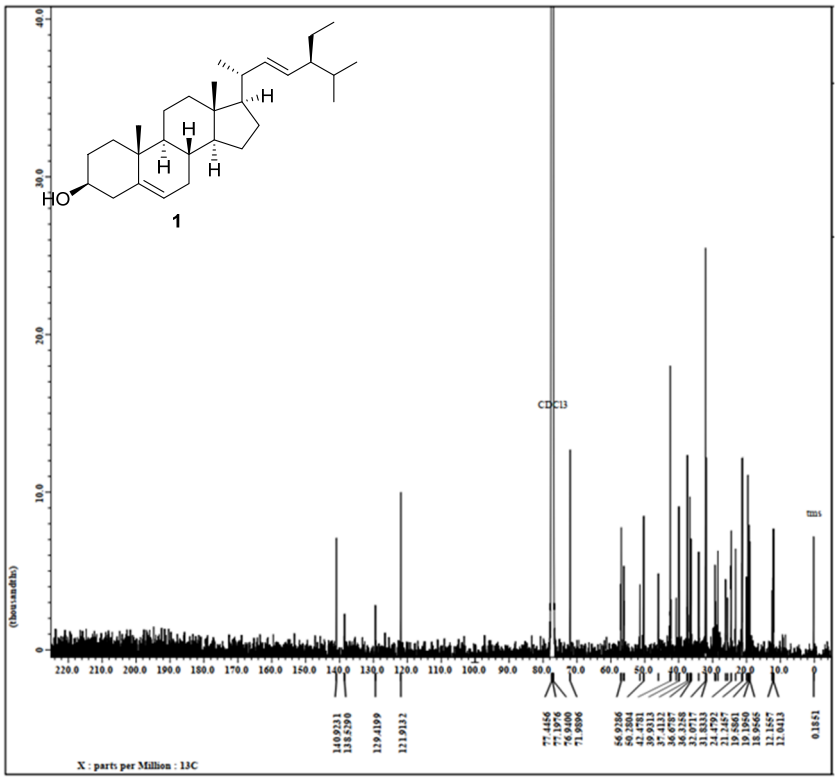
Nuclear Magnetic Resonance (NMR) of Isolated Compound 1-2



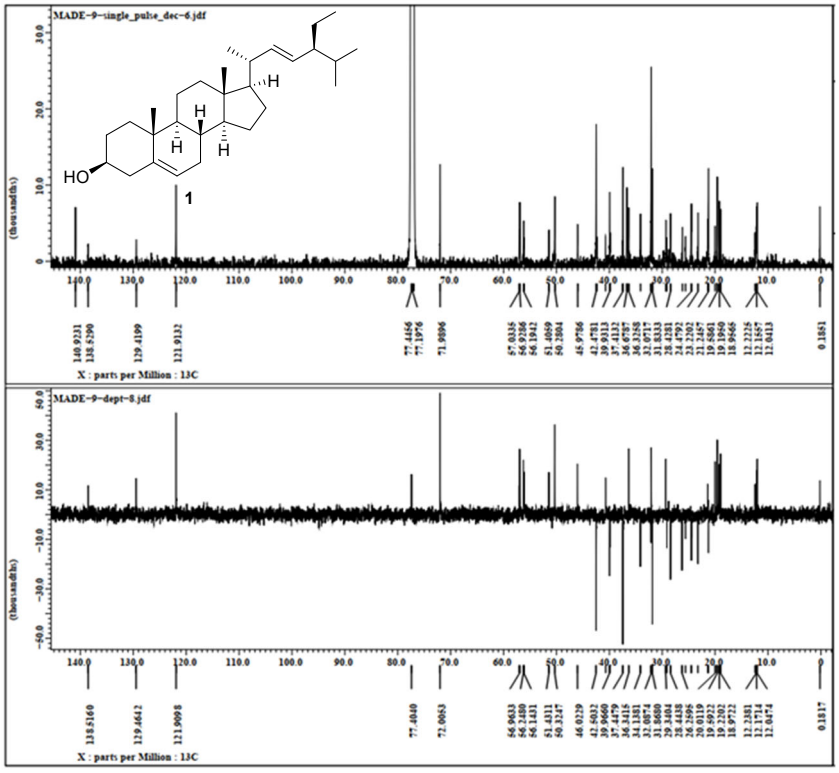
^{1}H-NMR ($CDCl_3$, 500 MHz)



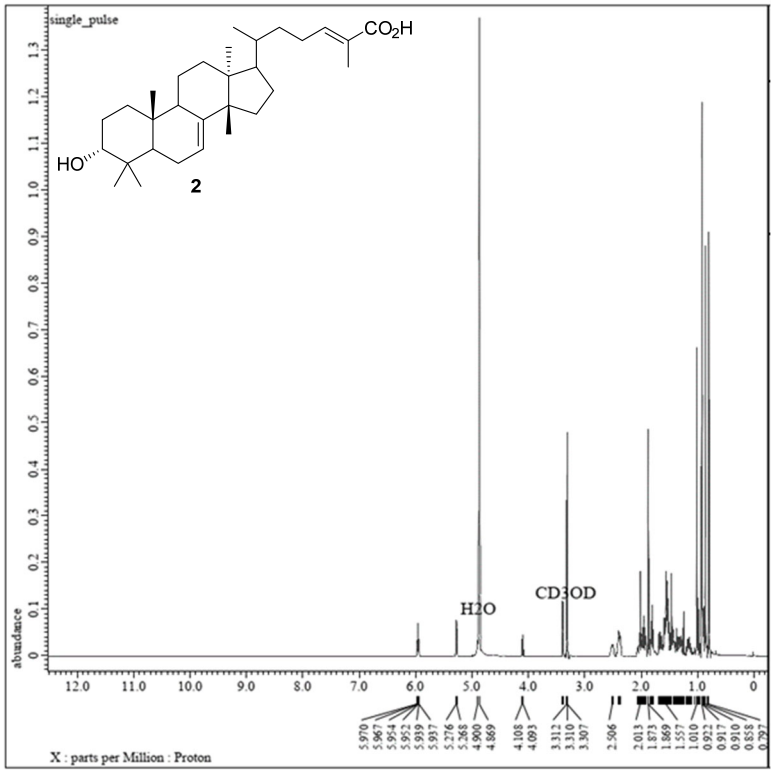
^{1}H-NMR-Expansion ($CDCl_3$, 500 MHz)



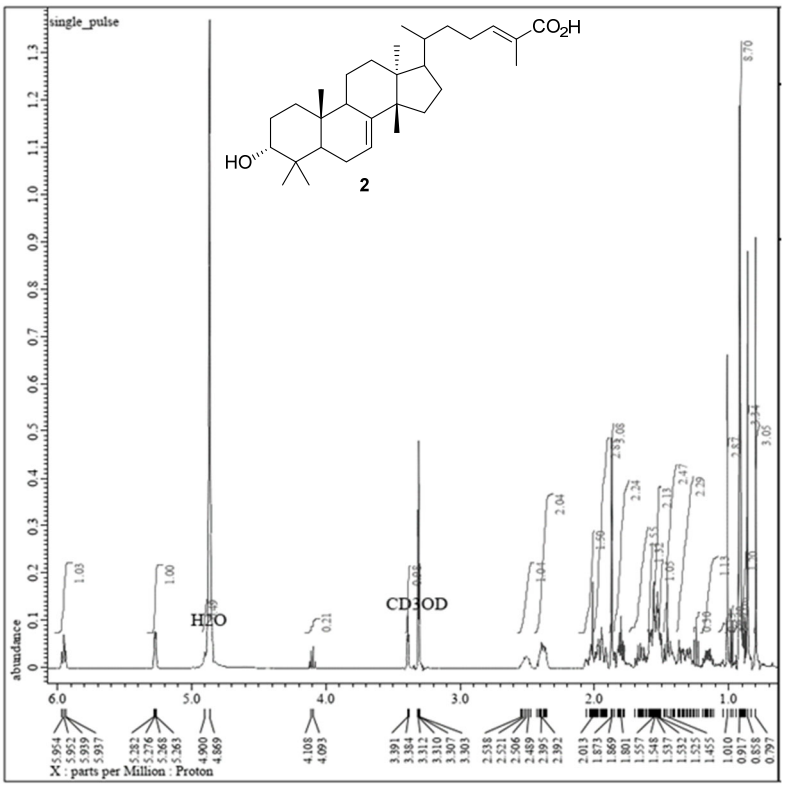
^{13}C-NMR ($CDCl_3$, 125 MHz)



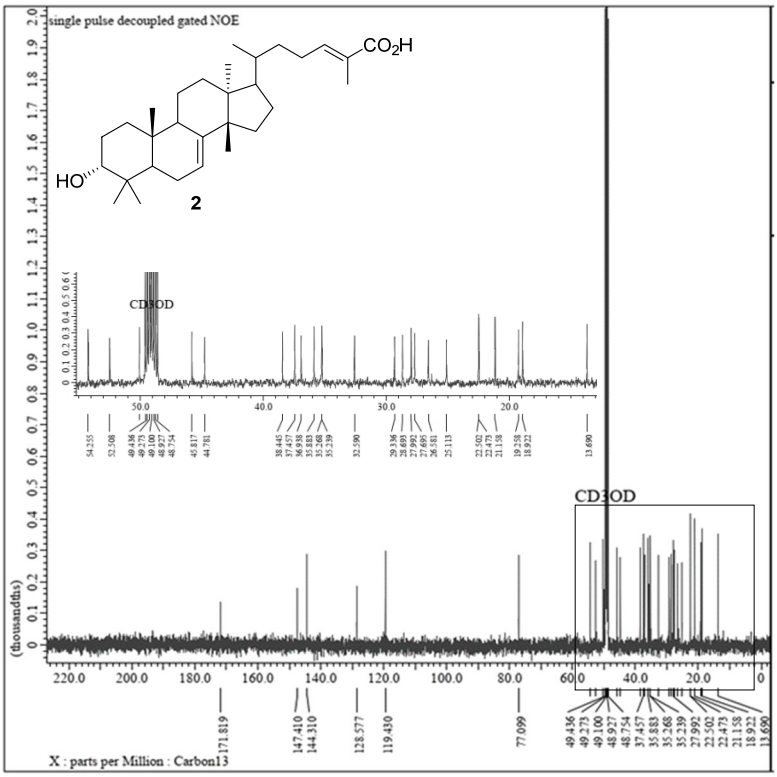
^{13}C-NMR and DEPT 135 ($CDCl_3$, 125 MHz)



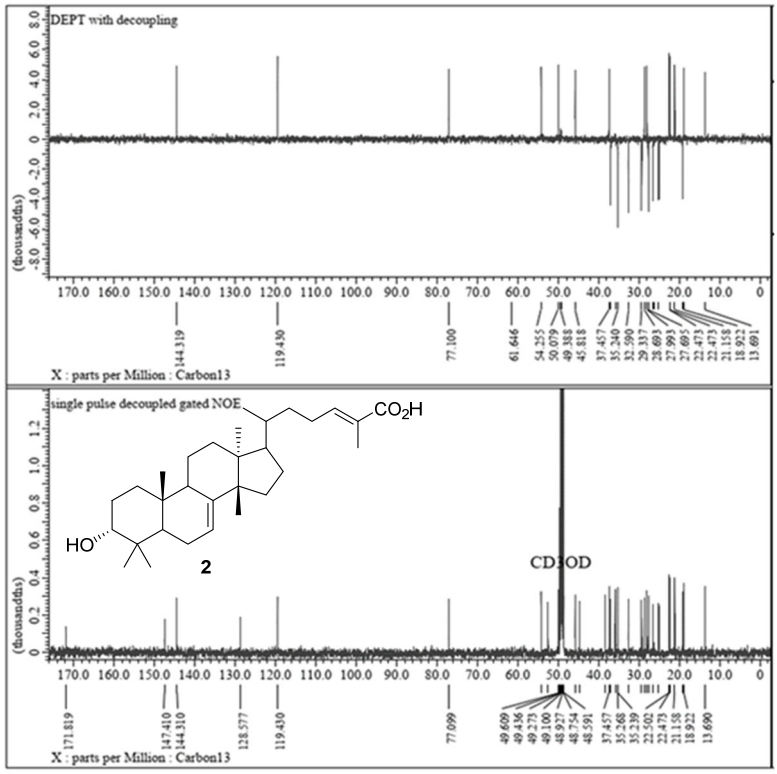
^{1}H-NMR (CD_3OD, 500 MHz)



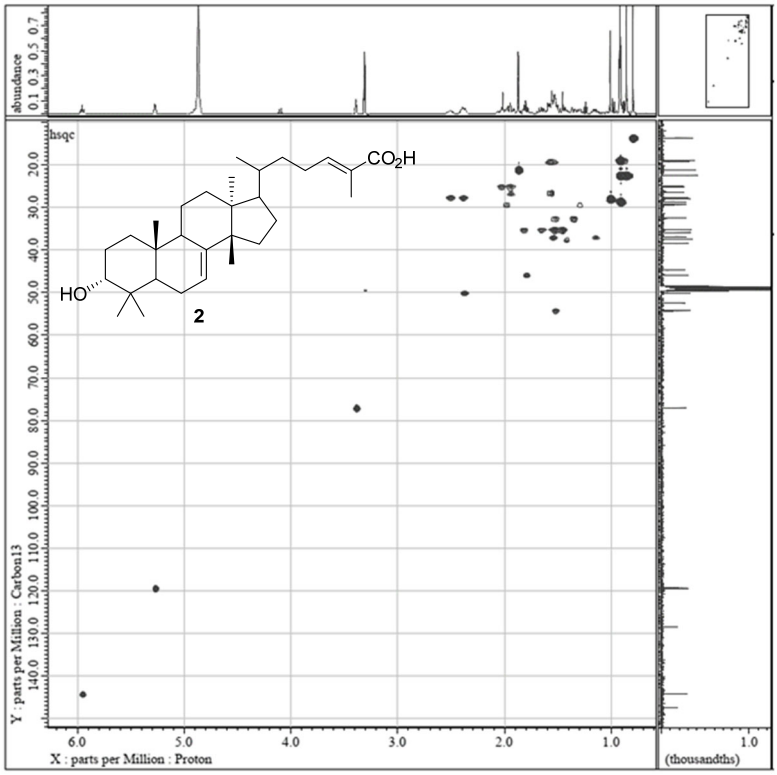
^{1}H-NMR-Expansion (CD_3OD, 500 MHz)



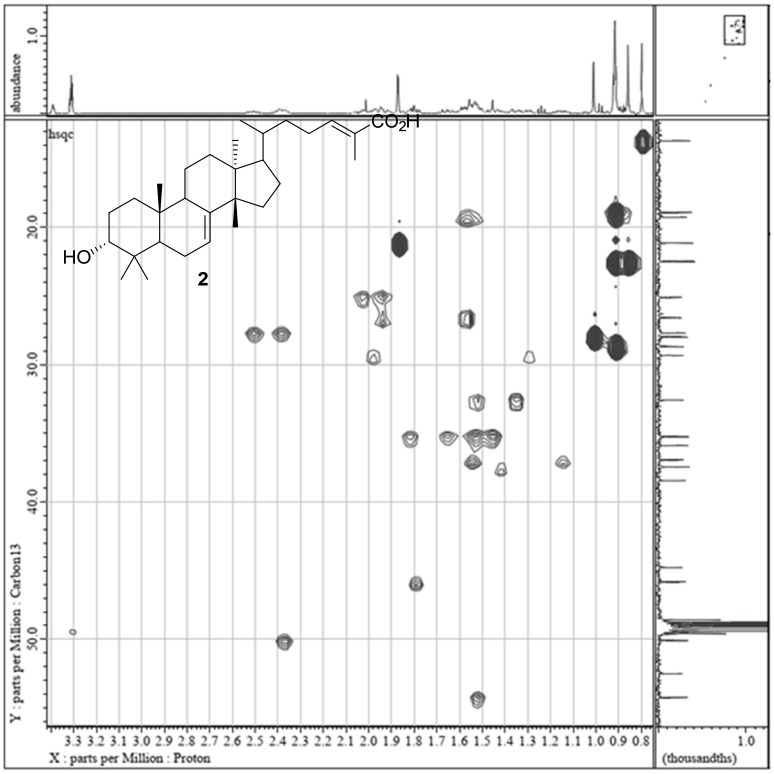
^{13}C-NMR (CD_3OD, 125 MHz)



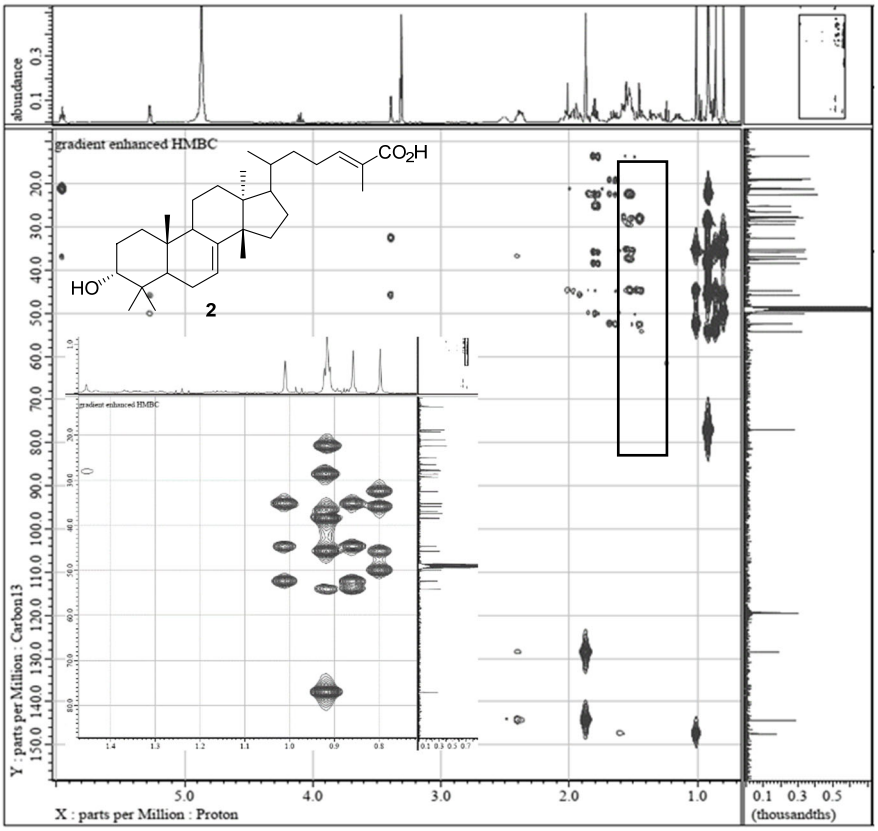
DEPT 135 and ^{13}C-NMR (CD_3OD, 125 MHz)



HSQC NMR (CD_3OD, 500 MHz)



HSQC NMR (Expansion, CD_3OD, 500 MHz)



HMBC NMR (CD_3OD, 500 MHz)

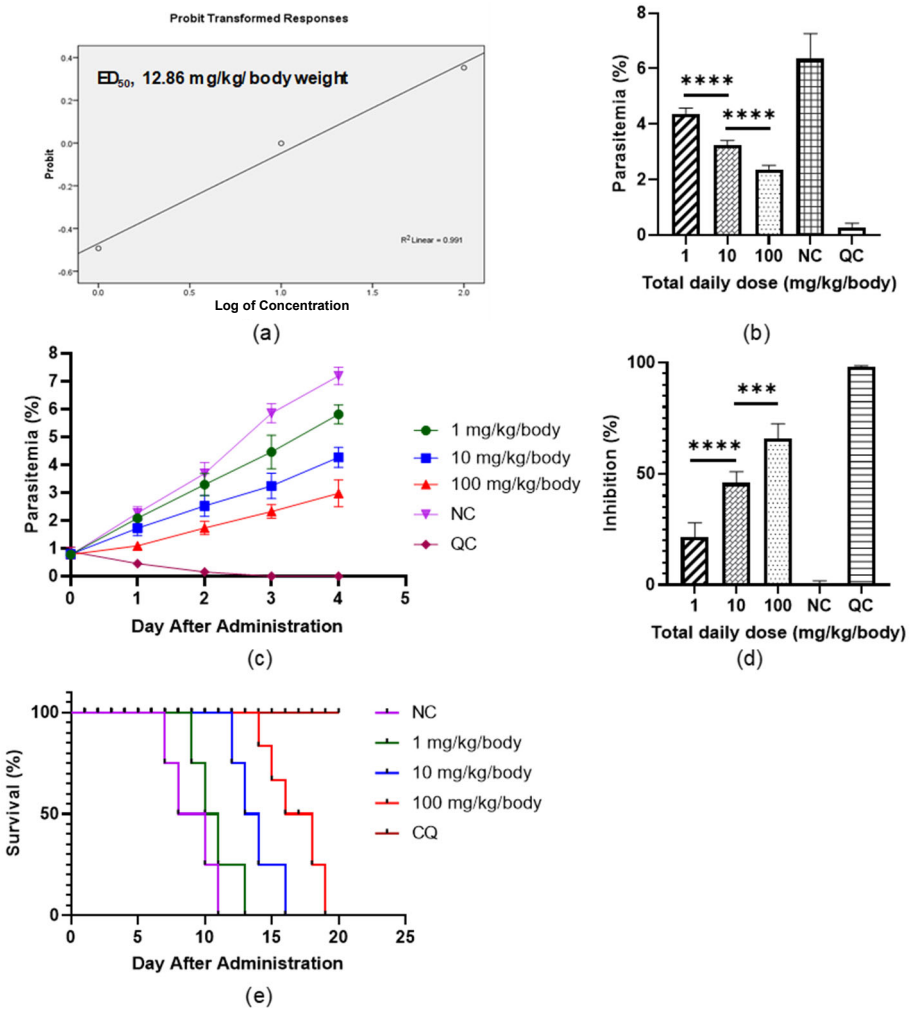


Fig S1. *In vivo* test of the PP extract against malarial parasite *Pb* ANKA strain in BALB/c albino mice. (a) ED_{50} value of the PP extract in mice. (b) Parasitemia rates after the fourth day of treatment. (c) Chemosuppression of parasitemia from day 0 to day 4. (d) Inhibition rates after the fourth day of treatment. (e) Survival mice treated with 0.5% CMC-Na as a negative control, the PP extract at various doses (1, 10 and 100 mg/kg/body) and chloroquine as a positive control. Bars in Fig. S1(b) and S1(d) indicate the parasitemia and parasite growth inhibition rates treated with 0.5% CMC-Na (NC) (grid), CQ (horizontal) and the PP extract at a daily dose of 1 mg/kg/body (pencil striped), 10 mg/kg/body (herringbone) and 100 mg/kg/body (halftone), while in Fig. S1(c) and S1(e) indicate parasitemia reduction from day 0 to day 4 and survival of mice treated with 0.5% CMC-Na (pink), CQ (carmine) and the PP extract at a daily dose of 1 mg/kg/body (green), 10 mg/kg/body (blue) and 100 mg/kg/body (red). SD is indicated by the error bars. ***$p < 0.001$, ****$p < 0.0001$

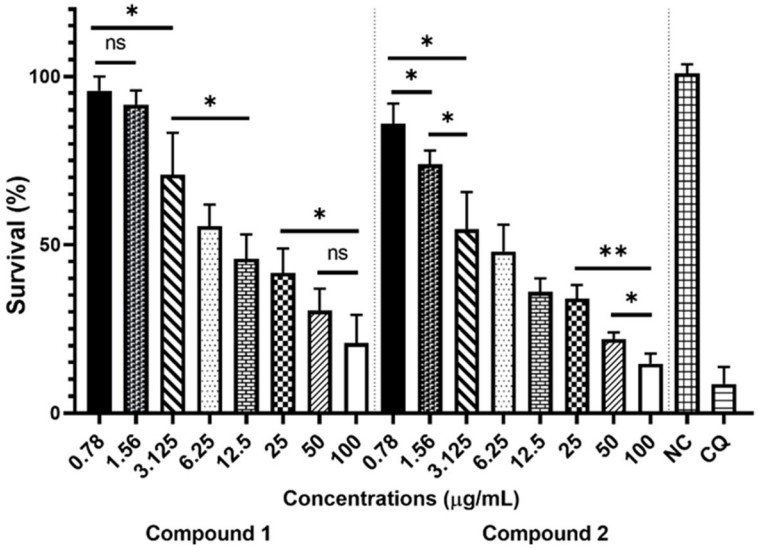


Fig S2. Survival rates of untreated parasites (0.2% DMSO) and parasites treated with compounds **1**–**2** at a concentration of 0.78 to 100 μg/mL and CQ (1 μM) are shown. Bars indicate parasite survival rates. Untreated parasites (NC - grid), CQ (horizontal) and compounds **1**–**2** at a concentration of 100 μg/mL (white), 50 μg/mL (small striped), 25 μg/mL (checkerboard), 12.5 μg/mL (brick), 6.25 μg/mL (halftone), 3.125 μg/mL (big striped), 1.56 μg/mL (hexagon) and 0.78 μg/mL (black). All experiments were performed in triplicate ($n = 3$). Standard Deviation (SD) is indicated by the error bars. *$p < 0.05$, **$p < 0.01$, not significant (ns)

Statistical analysis

Experiments were performed independently in triplicate and average are presented. Statistical analysis was performed by unpaired two-tailed *t*-test using GraphPad Prism. The statistics were significant when *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$

Table S1. *In vitro* Antiplasmodial activity of plant extract against *Plasmodium falciparum* [3]

IC_{50} value (μg/mL)	**Category of activity**
< 10	Promising
10–20	Moderate
20–40	Good
40–70	Marginally potent
> 70	Poor

References

[1] Greca, M.D., Monaco, P., and Previtera, L., 1990, Stigmasterols from *Typha latifolia*, *J. Nat. Prod.*, 53 (6), 1430–1435.

[2] Isaka, M., Chinthanom, P., Sappan, M., Supothina, S., Vichai, V., Danwisetkanjana, K., Boonpratuang, T., Hyde, K.D., and Choeyklin, R., 2017, Antitubercular activity of mycelium-associated *Ganoderma* lanostanoids, *J. Nat. Prod.*, 80 (5), 1361–1369.

[3] Kamaraj, C., Kaushik, N.K., Mohanakrishnan, D., Elango, G., Bagavan, A., Zahir, A.A., Rahuman, A.A., and Sahal, D., 2012, Antiplasmodial potential of medicinal plant extracts from Malaiyur and Javadhu hills of South India, *Parasitol Res.*, 111 (2), 703–715.