Supporting Information to:

Antiplasmodial Constituents from the Fruit Pericarp of

*Pentadesma butyracea*

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**Extraction and isolation**
The pericarp of the fruits of *P. butyracea* was separated from the seeds and dried separately. The ground pericarp (3.2 kg) was extracted at room temperature with a mixture of CH$_2$Cl$_2$–MeOH 1:1 (2 × 5 L, 24 h each). The solvent was evaporated under reduced pressure to yield 102 g of extract. The ground seeds (2.3 kg) were also extracted at room temperature with CH$_2$Cl$_2$–MeOH 1:1 (2 × 3 L, 24 h each). The solvent was removed under reduced pressure to yield 57 g of extract. The two extracts then were screened for their antiplasmodial activity; the pericarp extract showed good potency, while the seed extract was inactive.

A portion of 80 g of the pericarp extract was fractionated by flash chromatography over silica gel (70–230 mesh, 7 × 42 cm; Merck), eluted with a gradient of increasing polarity of mixtures of hexane–ethyl acetate 100:0–0:100, resulting in the collection of 80 fractions of 500 mL each, which were combined on the basis of TLC analysis to yield three main fractions: P1–P3. Fraction P1 (35.0 g, composed of subfractions 1–28) was a complex mixture containing essentially oil and thus was not studied. Fraction P2 (17.1 g, composed of subfractions 29–53) was subjected to column chromatography over silica gel (230–400 mesh, 5 × 42 cm; Merck), eluted with gradient mixtures of hexane–ethyl acetate 100:0–80:20, resulting in 122 subfractions of 200 mL, combined on the basis of TLC analysis into three fractions: P21, P22, and P23.

Fraction P21 (9.2 g, composed of subfractions 1–47) was separated by column chromatography over silica gel (230–400 mesh, 4 × 40 cm; Merck), eluted with hexane–ethyl acetate 90:10, to yield lupeol (7) (1300 mg). Fraction P22 (4.5 g, composed of subfractions 48–81) was separated by column chromatography over silica gel (230–400 mesh, 5 × 42 cm; Merck), eluted with hexane–ethyl acetate 80:20, to yield garcinone E (5) (50 mg) and α-mangostin (3) (22 mg). Fraction P23 (3.6 g, composed of subfractions 82–122) was separated by column chromatography over silica gel (230–400 mesh, 3.5 × 30; Merck), eluted with hexane–ethyl acetate 80:20–70:30, to yield pentadexanthone (1) (7 mg), 1,3,5-trihydroxy-2-methoxyxanthone (4) (10 mg), and cratoxylone (2) (40 mg). Fraction P3 [26.1 g, composed of subfractions 54–80 (500–13,000 mL)] was subjected to column chromatography over silica gel (230–400 mesh, 5 × 42; Merck), eluted with n-hexane–EtOAc mixture 70:30, to yield epicathexatin (6) (35 mg). The seed extract, which was inactive on the plasmodium falciparum W2 strain, was not further studied.
Pentadexanthone (1): Yellow powder (n-hexane); Rf = 0.6, silica gel 60 F254, hexane–ethyl acetate (70:35); UV (MeOH): $\lambda_{\text{max}} = 236, 260, 276, 313, \text{and } 370 \text{ nm}$; $^1\text{H}$- and $^{13}\text{C}$-NMR data: see Table 1 in main text; EI-MS: $m/z = 424$ (76), 409 (49), 406 (38), 391 (32), 363 (40), 183 (18); HR-ESI-MS: $m/z = 423.144889 \ [M–H]^+$ (calcd. for C$_{24}$H$_{23}$O$_7$: 423.14439).

**Biological evaluation**

This evaluation consisted of two steps: 1) a preliminary test of erythrocyte susceptibility to compounds and extracts and 2) a screening for antiplasmodial activity.

**Evaluation of erythrocyte susceptibility to compounds in vitro**

A preliminary toxicological assessment was carried out to determine the highest drug concentrations that can be incubated with erythrocytes without any significant damage. This was done according to the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide/phenazine methosulfate (MTT/PMS) colorimetric assay described by Cedillo-Rivera et al. with some modifications [1]. The drugs were serially diluted in 96-well culture plates, and each concentration was incubated in triplicate with erythrocytes at 2% hematocrit in a final culture volume of 100 $\mu$L (at 37 °C in an atmosphere of 3% O$_2$, 5% CO$_2$, and 91% N$_2$, in the presence of RPMI 1640 medium and 25 mM HEPES, pH 7.4, for 48 h). At the end of the incubation period, the cultures were transferred into polypropylene microcentrifuge tubes and centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. MTT solution (1.5 mL) and 250 $\mu$g PMS were added to the pellets. Controls contained no erythrocytes. The tubes were thereafter incubated for 45 min at 37 °C and then centrifuged, and the supernatant was discarded. The pellets were re-suspended in 0.75 mL of 0.04 M HCl in isopropanol to extract and dissolve the dye (formazan) from the cells. After 5 min, the tubes were vigorously mixed and centrifuged, and the absorbance of the supernatant was determined at 570 nm. More formazan (highest O.D.) was produced in tubes containing the most viable cells.

**Evaluation of the antiplasmodial activity**

*Plasmodium falciparum* strain W2, which is resistant to chloroquine and other antimalarial drugs [2], was cultured in sealed flasks at 37 °C (in an atmosphere of 3% O$_2$, 5% CO$_2$, and 91% N$_2$) in RPMI 1640 medium and 25 mM HEPES, pH 7.4, supplemented with heat-inactivated 10% human serum and human erythrocytes to achieve a 2% hematocrit. Parasites
were synchronized in the ring stage by serial treatment with 5% sorbitol (Sigma) [3] and studied at 1% parasitemia.

Stock solutions of plant products were prepared as 1 mg/mL in DMSO, diluted as needed for individual experiments, and tested in triplicate. The stock solutions were diluted in supplemented RPMI 1640 medium so as to have at most 0.2% DMSO in the final reaction medium. An equal volume of 1% parasitemia, 4% hematocrit culture was thereafter added and gently mixed thoroughly. Negative controls contained equal concentrations of DMSO. Positive controls contained 1 μM chloroquine phosphate (Sigma). Cultures were incubated at 37 °C for 48 h (1 parasite erythrocytic life cycle).

Parasites at ring stage were thereafter fixed by replacing the serum medium with an equal volume of 1% formaldehyde in PBS. Aliquots (50 μL) of each culture were then added to 5-mL round-bottom polystyrene tubes containing 0.5 mL 0.1% Triton X-100 and 1 nM YOYO nuclear dye (Molecular Probes) in PBS. Parasitemias of treated and control cultures were compared using a Becton-Dickinson FACSort flow cytometer to count nucleated (parasitized) erythrocytes. Data acquisition was performed using CellQuest software. These data were normalized to percentage control activity and 50% inhibitory concentrations (IC50) calculated using Prism 5.0 software (GraphPad) with data fitted by non-linear regression to the variable slope sigmoidal dose-response formula $y = 100 / [1 + 10^{(\log IC50 - x)/H}]$, where $H$ is the hill coefficient or slope factor [2].

References