Supporting Information

_In Vitro_ Antiplasmodial Activity of Benzophenones and Xanthones from Edible Fruits of _Garcinia_ Species

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Extraction and partitioning of plant material

Initial extraction of *Garcinia* fruits was performed on deseeded, lyophilized fruit pulp and skin. The fruit was homogenized in a blender with 8:2 MeOH:H₂O and extracted overnight twice at room temperature. The resulting crude MeOH extracts were combined, filtered and concentrated *in vacuo* at 35-40°C. A large-scale extraction of *G. xanthochymus* seeds separated from the fruit pulp was performed by Naturex. The seeds were pulverized then homogenized with 80% aqueous EtOH. This crude EtOH extract was then dried to 66.36% solids *in vacuo* at temperatures not exceeding 40°C.

A sample of the resulting semi-dry seed extract was redissolved into 8:2 MeOH:H₂O, filtered, and then partitioned using three separate solvent-solvent partitioning schemes (Fig. 1S). The resuspended residue was sequentially partitioned with hexanes, EtOAc, and n-BuOH, or CHCl₃, EtOAc, and n-BuOH, or hexanes, CHCl₃, and n-BuOH. The resulting partitions were dried *in vacuo* at 35-40°C and evaluated via HPLC-PDA. Based on the HPLC-PDA analysis, a modified Kupchan scheme using hexanes, EtOAc, and n-BuOH was chosen to partition the remaining crude EtOH seed extract. The extract was partitioned as described above, and the resulting partitions were dried *in vacuo* at 35-40°C to yield three dried residues for further study. The dried hexanes, EtOAc, and BuOH residues were submitted to antiplasmodial testing.
Solvent-solvent partitioning

The *G. xanthochymus* ethanolic seed extract underwent several solvent-solvent partitioning schemes in order to identify a suitable method (Figs. 1S and 2S). Using RP HPLC-PDA the solvent-solvent partitioning of hexanes, EtOAc, and n-BuOH was chosen for further phytochemical analysis as it was determined to provide the best separation of compounds between the three partitions with the least amount of overlap from one partition to the other. In this optimized partitioning scheme, 6.99 g of the *G. xanthochymus* seed extract was sequentially partitioned with: hexanes, EtOAc, and n-BuOH. The combined yield of the partitions was 5.19 g or 74% of the initial extract. In order to produce a strong solvent line, the proportion of water was increased in the aqueous phase for each partition.

Chromatograms for each of the *G. xanthochymus* partitions were evaluated by RP HPLC-PDA. The chromatogram of the hexanes partition showed most of the compounds eluting off the column at around 25 min, at a high MeCN concentration, and these peaks were determined to be mostly benzophenones by examination of their UV-VIS spectra and later HPLC-spiking experiments. The EtOAc chromatogram had some extremely hydrophilic compounds eluting in the first 5 min of the run with 90% aqueous solvent. The majority of the compounds in the EtOAc partition eluted off the column between 10 and 20 min. Based on the UV-VIS spectra, these were identified as mid-polar biflavonoids and biflavonoid glycosides eluting at nearly 50:50 10 mM aqueous ammonium acetate:MeCN. Due to the presence of the sugar moiety, the biflavonoid glycosides elute slightly earlier, at a higher aqueous concentration, than the less polar
biflavonoids. Lastly, the n-BuOH partition showed the earliest eluting hydrophilic compounds that were seen in the EtOAc partition. The presence of these similar hydrophilic compounds leads to the conclusion that they were carried over from the aqueous phase. The chromatogram from the n-BuOH partition also had some peaks eluting around 15 min, which were determined to be biflavonoid glycosides, based on their UV-VIS spectra.

**Chromatography and isolation of compounds**

Using the optimized solvent-solvent partitioning scheme, the dried hexanes residue (30 g) was suspended in MeOH using sonication. The material was then chromatographed in a 105 x 2.5 cm open column over Sephadex LH-20 (200 g, dry weight) and eluted with MeOH. The resulting 18 fractions, A-R, were analyzed by RP TLC. The plate was examined under UV light and then developed with a vanillin spray reagent. Based on the RP TLC analysis similar fractions were combined and dried in vacuo. The recombined fractions were analyzed by RP HPLC-PDA. The total yield of all the fractions was 27.4 g, which is 90.4% of the starting material.

The dried EtOAc residue (41 g) was redissolved in MeOH and also chromatographed over Sephadex LH-20 (200 g) eluted with MeOH. The resulting 66 fractions, A-NNN, were analyzed by RP TLC and developed with a vanillin spray reagent. The RP TLC analysis allowed the fractions to be recombined into 11 fractions. The recombined fractions were dried in vacuo and analyzed by RP HPLC-PDA Purified standards were used to verify the identity of isolated compounds via HPLC spiking experiments.
Several compounds were isolated from the methanolic extract of *G. xanthochymus* fruit pulp. The extract was partitioned with CHCl₃ and EtOAc. The CHCl₃ partition underwent open column chromatography with Sephadex LH-20 and then open column chromatography with RP C₁₈ before being subjected to preparative RP HPLC to purify alloathyriol. Additionally Sephadex LH-20 and RP open column chromatography of the CHCl₃ partition yielded aristophenone A, cycloxanthochymol, gambogenone A, guttiferone E, guttiferone H, isoxanthochymol, and xanthochymol. In order to separate the double bond isomer mixture of cycloxanthochymol and isoxanthochymol, and the mixture of guttiferone E and xanthochymol, argentation TLC was necessary. The biflavonoids and their glycosides amentoflavone, fukugetin, fukugiside, and volkensiflavone were also isolated from the EtOAc partition. The EtOAc partition repeatedly underwent open column chromatography with Sephadex LH-20 and preparative RP HPLC to purify these compounds.

Guttiferone A was isolated from a MeOH extract of the fruit pulp of *G. livingstonei*. The extract was partitioned with H₂O, EtOAc, and n-BuOH. The EtOAc partition was repeatedly chromatographed over Sephadex LH-20 via open column, and preparative RP HPLC was used to purify guttiferone A.

The benzophenone 32-hydroxy-ent-guttiferone M was isolated from the methanolic seed extract of *G. edulis*. The extract was partitioned with CHCl₃, EtOAc, and n-BuOH. The EtOAc was chromatographed over a RP C₁₈ open column and then subjected to preparative RP HPLC.

Mangiferin was isolated from the leaves of *Phaleria nisidai*. The leaves were exhaustively extracted with MeOH under reflux. The extract was dried *in vacuo* and then
dissolved in H₂O and CHCl₃; mangiferin precipitated out of the solution. The precipitate was filtered and then washed with CHCl₃ and MeOH.

The purity of purchased standards was verified via RP HPLC-PDA. Chromatograms for the xanthone and xanthone glycoside standards used for this research, including 3-isomangostin, α-mangistin, β-mangostin, 4-methoxy-9H-xanthone, 1,5,6-trihydroxyxanthone, and xanthene-9-one are shown in Fig. 3S. Chromatograms used to evaluate the purity of the benzophenones and biflavonoids are shown in Fig. 4S and 5S, respectively. The same HPLC-PDA method and gradient system was used for determining the purity of these standards as for the *G. xanthochymus* fractions.

**Column and TLC chromatography**

Open column chromatography was done over the size-exclusion matrix, Sephadex LH-20 and eluted with MeOH. The resulting fractions were dried *in vacuo* at 35-40°C. TLC analysis was performed using RP TLC plates with either 1:3 10 mM ammonium acetate:MeCN or 1:1 10 mM ammonium acetate:MeCN as the mobile phase. The plates were examined under UV light and then developed with a vanillin spray reagent composed of 1% vanillin in 10% ethanolic sulfuric acid.

**HPLC methods**

HPLC analyses of samples was performed on a Waters 2695 Separations Module equipped with a Waters 996 or 2996 photodiode array detector and monitored using Waters Empower 2 software (build 2154). The analysis was performed with either a Phenomenex Nucleosil RP C₁₈ column (250 x 4.6 mm, 5 µM) or a Phenomenex Synergi...
Hydro RP C$_{18}$ (250 x 4.6 mm, 4 μM) column. The HPLC samples were prepared in HPLC grade MeOH and passed through a syringe filter prior to HPLC analysis.

Several HPLC solvent systems were evaluated to optimize the chromatographic separation. The gradient profile of the method used 10 mM ammonium acetate (solvent A) and MeCN (solvent B) and was as follows: 0-4, 90% A, 4-34 min, 90-0% A; 34-44 min, 0% A; concluding with 10 min to reestablish initial conditions and equilibrate the column to 90% A.

The slow return to initial conditions was only necessary when using the Synergi Hydro column in order to prevent the HPLC system from going over pressure. Additional HPLC experiments indicated that additional run time were necessary when using the Synergi Hydro column.
Fig. 1S Solvent-solvent partition scheme used with *G. xanthochymus* seed extract.
Fig. 2S HPLC-PDA chromatograms for solvent-solvent partitioning scheme used with *G. xanthochymus* seed extract: hexanes (a), EtOAc (b), and n-BuOH (c). All chromatograms are shown at 320 nm, from a Synergi column with a solvent system of 10mM ammonium acetate (A) and MeCN (B). Initial conditions were set at 90A:10B.
Fig. 3S RP HPLC-PDA chromatograms and UV spectra of xanthones and xanthone glycosides used in this study: (a) alloathyriol, (b) 8-desoxygartanin, (c) 3-isomangostin, (d) mangiferin, (e) α-mangostin, (f) β-mangostin, (g) 4-methoxy-9H-xanthone, and (h) 1,5,6-trihydroxyxanthone. All chromatograms extracted at 254 nm.
Fig. 4S RP HPLC-PDA chromatograms and UV spectra of benzophenones used in this study, (a) aristophenone A, (b) cycloxyanthochymol, (c) gambogenone, (d) guttiferone A, (e) guttiferone E, and (f) guttiferone H. All chromatograms extracted at 254 nm.
Fig. 5S  
RP HPLC-PDA chromatograms and UV spectra of biflavonoids used in this study: (a) fukugetin, (b) fukugiside, and (c) volkensiflavone. All chromatograms extracted at 254 nm.
hexanes extract
30.35 g

Stationary Phase: ~200 g LH-20
Mobile Phase: 100% MeOH

Fractions
A - C

Fractions
D - E

Fraction
F

Fractions
G - H

Fractions
I - N

Fraction
O

Fractions
P - R

0.0238 g

0.0783 g

0.7169 g

2.8015 g

23.25 g

0.4551 g

0.1053 g

Cmpds
3, 4, 5, 6, 7, 8

Fig. 6S Fractions, yields, and resulting compounds from the hexanes partition of the G. xanthochymus seeds.