Supporting Information to:

Antioxidant and Hepatoprotective Activities of Thai Mango Seed Kernel Extract

Saruth Nithitanakool¹, Pimolpan Pithayanukul¹, Rapepol Bavovada²

Affiliation
¹ Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
² Department of Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

Correspondence
Associate Professor Dr. Pimolpan Pithayanukul
Department of Pharmacy
Faculty of Pharmacy
Mahidol University
Bangkok 10400
Thailand
Phone/Fax: +662/6/448 694
pypph@mahidol.ac.th, pypph@hotmail.com
Materials and Methods

Extraction and isolation of constituents
Fresh seeds were washed and the kernels (4.75 kg) were removed manually from the seeds, chopped and promptly homogenized in a blender. Hot ethanol (80 °C) was used as the extracting solvent, at a ratio of seed kernels/solvent of 1:2 (w/v), for 10 min at room temperature (30°C) prior to centrifugation at 2000 rpm for 15 min in a Hattich Roto magna®. The extraction was performed three times. The ethanol extracts were filtered, combined and concentrated in a rotary evaporator (BÜCHI Rotavapor R-200) at 40°C. The extract was defatted with hexane, evaporated under reduced pressure, and then freeze-dried to afford a crude mango seed kernel extract (MSKE) with a yield of 411.35 g (8.66 % w/w).

The dried MSKE (10 g) was subjected to column chromatography using a polyamide 11 (300 g, Ø 7 × 100 cm), eluted with 4 L of CHCl₃/MeOH/EtOAc (1:0.7:2.5), to give 0.16 g of methyl gallate (1) together with three major fractions (I-III). Fraction II (0.3 g) was subjected to a silica gel 60 column (40–63 µm, 5 g, Ø 1.5 × 59 cm), which was eluted with 400 mL of CHCl₃/EtOH/EtOAc/ethyl methyl ketone (3:0.5:1:1) to afford 0.11g of gallic acid (2).

Fraction III (3.1g) was chromatographed on a silica gel 60 column (40–63 µm, 30g, Ø 5 × 30 cm) eluted with 1.8 L of CHCl₃/EtOH/EtOAc/ethyl methyl ketone (3:0.5:1:1) followed by a Sephadex LH-20 (Ø 2.5 × 30 cm) eluted with 1 L of acetone, to give 1.25g of 1,2,3,4,6-penta-O-galloyl-β-D-glucopyranose (3).

Standardization
Calibration curves for each compound (1–3) were obtained by densitometric scanning of different quantities of the chemical marker bands on developed chromatographic plates. An aliquot of the crude extract (MSKE) (8 µL, 25 mg/mL) was applied along with serial amounts of the chemical marker stock solution. The thin-layer chromatographic (TLC) plates were developed in a pre-saturated twin trough glass tank using CHCl₃/MeOH/EtOAc/ethyl methyl ketone (6:1.6:2:2) with five drops of formic acid as the mobile phase for compounds 1–2 and CHCl₃/EtOH/formic acid (3:5:1) for compound 3. The developed TLC plates were scanned at 286 nm and the amount of each compound (GA 4.4, MG 6.8 and PGG 612.8 mg/g dry weight) in MSKE was calculated from the calibration curves.