Supplementary file 1

Phytochemical profiling, antioxidant potential, and anti-*Acinetobacter baumannii* activity of *Macadamia integrifolia* Maiden & Betche shell extracts

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Instruments

A sonicator (40 kHz, PS-80A, China) and a rotary evaporator (Onilab, USA) were used in sample preparation. A rotary evaporator (Onilab RE100-PRO, USA) and a microscope (Olympus, Thailand) were used for the microscopic study. Linomat 5 sample applicator (Camag, Switzerland) and a TLC visualizer 3 (Camag, Switzerland) were used to spot the samples in TLC plates and observe the TLC fingerprint, respectively. The high-performance liquid chromatography (HPLC) system (Shimadzu pump LC-10ATvp, Japan) was used to obtain an HPLC chromatogram. The orbital shaker (Topscien, Singapore) was used to mix the reaction mixture in a 96-well plate assay. A microplate reader (BioTek, USA) and UV-spectrophotometer (Model UV-1800, Shimadzu, Japan) were employed for analytical measurements. The incubator (Shel Lab, USA) incubated all microbes. The biosafety cabinet class II (AC2-4S8-NS, Esco, Thailand) was used for handling biological samples under sterile conditions. An autoclave (Model ES-315, TOMY, high-pressure steam sterilization, USA) was used for sterilization.

Plant extraction

Macadamia shell powder was extracted with 95% ethanol (1:5 ratio of powder to ethanol), followed by ultrasonication for 30 minutes at room temperature (30±10°C). The extraction was repeated three times. The combined supernatants were evaporated at 50°C under reduced pressure to obtain a crude extract (ME). For liquid-liquid partitioning, 100 g of ME was mixed with 200 mL deionized water and 400 mL dichloromethane, sonicated for 5 minutes, separated the dichloromethane layer from the water layer, and repeated these processes three times. The dichloromethane layers from three times fractionation were combined and evaporated at 50°C under reduced pressure to yield the dichloromethane fraction (MD). The remaining water layer was subsequently partitioned with 400 mL of ethyl acetate using the

same protocol as dichloromethane to obtain the ethyl acetate fraction (MA). The remaining water layer was evaporated at 50°C under reduced pressure, then freeze-dried to obtain the water fraction (MW). All fractions (ME, MD, MA, and MW) were stored at 4 ± 3°C until analysis.

Quantitative analysis of *Macadamia integrifolia* shell extracts

Total phenolic content determination

Determining total phenolic content in four fractions of *M. integrifolia* shell (ME, MD, MA, and MW) was conducted following Sembiring EN et al and Roy et al (17,18), with minor adjustments. The 25 µL of the tested samples (ME, MA, and MW (2.0 mg/mL), and MD (4.0 mg/mL) in methanol) were mixed with 100 µL of Folin–Ciocalteu reagent (diluted 1:4 in water) in a flat-bottom 96-well plate. Then, the reaction mixture was shaken at 200 rpm for 1 minute using an orbital shaker, followed by adding 75 µL of a saturated sodium hydrogen carbonate solution (6% w/v), with continuous shaking for an additional 1 minute. Subsequently, the reaction mixtures were incubated at room temperature (25±2 °C) for 2 hours. Absorbances were recorded at 765 nm using a microplate reader. Gallic acid dilutions (0–14 µg/mL) were used to establish a calibration curve. Methanol was used as a reagent blank. The total phenolic contents of the samples reported in mg gallic acid equivalent (GAE) per gram of the extract.

Total flavonoid content determination

An Aluminum chloride assay described by Sembiring EN et al. and Ahmad et al. (17,19), with some modifications, was used to measure the total flavonoid content of the extract. Rutin solutions in methanol at 0 - 40 µg/ml concentrations were used to prepare a calibration curve. Methanol was used as a reagent blank. The 50 µl of four fractions of *M. integrifolia* shell extracts in methanol (1.0 mg/ml) or standard solution was mixed with 10 µl of 10% Aluminum chloride solution, then 130 µl of methanol in a 96-well plate. Sodium acetate

solution (1 M, 10 µl) was added to the reaction mixture. All reagents were mixed well and incubated for 40 minutes at room temperature, protected from light. The absorbance was measured at 415 nm using a microplate reader. Total flavonoid contents were calculated and expressed as mg rutin equivalent (RTE) per gram of the extract.

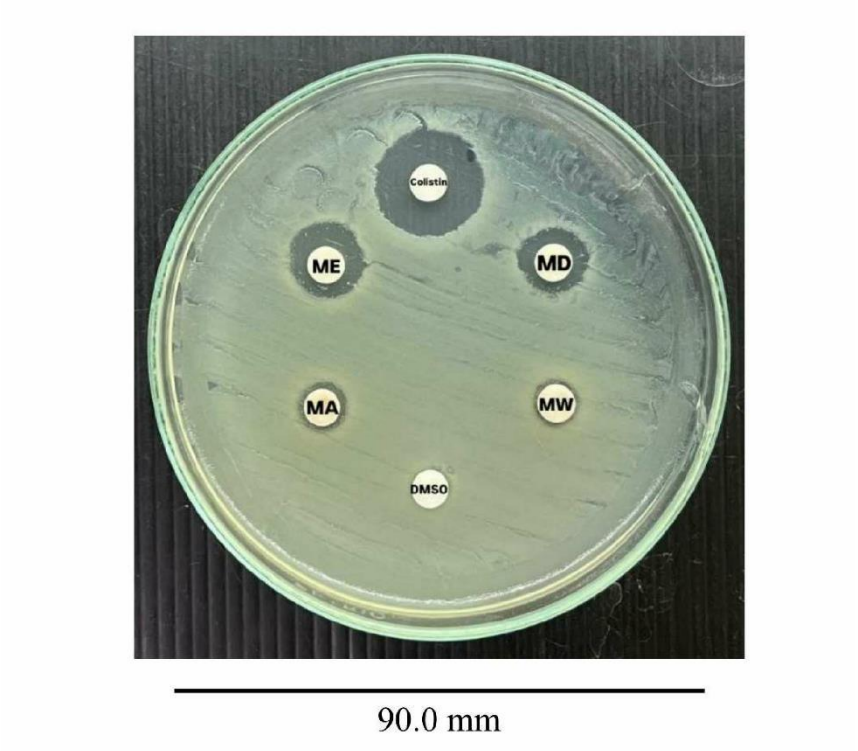

Figure S1. Growth inhibition zones of *Acinetobacter baumannii* (ATCC 19606) assessed using the paper-disc diffusion assay for four macadamia shell fractions: ME (ethanolic crude extract), MD (dichloromethane fraction), MA (ethyl acetate fraction), and MW (water fraction) at 10 mg/mL (20 µL/disc). Colistin (10 mg/mL, 20 µL/disc) was used as a positive control, and dimethyl sulfoxide (DMSO, 20 µL/disc) was a negative control.