

Supplementary Methods S1

Detailed Experimental Procedures

S1. Plant Identification (Determination Test)

Plant identification was performed using fresh plant specimens with intact morphological parts, including roots, stems, and leaves. The identification process was conducted in a laboratory by comparing the morphological characteristics of the samples with standard taxonomic descriptions available in the literature to confirm the botanical identity of *Muntingia calabura* L. and *Pluchea indica* L.

S2. Preparation of Simplisia

The preparation of simplisia included the following steps: collection of plant materials, wet sorting to remove contaminants, washing with running water, chopping into smaller pieces, drying at room temperature under shade, dry sorting, packaging, and storage. Kersen leaves were collected from Kotagede, Yogyakarta, while beluntas leaves were collected from Umbulharjo, Yogyakarta, Indonesia.

S3. Extract Preparation

Dried leaf samples were pulverized into powder. Extraction was carried out using 96% ethanol as solvent at a ratio of 1:10 (w/v). A total of 100 g of powdered sample was macerated in 1 L of 96% ethanol and homogenized using a shaker incubator for 4 hours, followed by maceration for 24 hours at room temperature. The extract was filtered and concentrated using a rotary evaporator at 50°C under reduced pressure. Final concentration was completed using a water bath until a viscous extract was obtained.

S4. Extract Standardization

Extract standardization included organoleptic evaluation (color, odor, and consistency) and determination of moisture content using a halogen moisture analyzer to ensure extract quality and stability prior to antibacterial testing.

S5. Phytochemical Screening

S5.1 Tube Tests

Alkaloid Test

A total of 10.5 g of extract was added to 2 mL of chloroform and 10 mL of ammonia solution, followed by the addition of 10 drops of sulfuric acid (H_2SO_4). The mixture was shaken and allowed to separate. The acidic layer was then treated with Dragendorff reagent. A positive result was indicated by the formation of a red-orange precipitate.

Flavonoid Test

A total of 0.5 g of extract was dissolved in 5 mL of ethanol and heated for 5 minutes. Subsequently, 10 drops of concentrated hydrochloric acid (HCl) and 0.2 g of magnesium powder were added. A positive reaction was indicated by the formation of a brownish-red color or precipitate.

Phenolic Compound Test

A total of 0.5 mL of extract was mixed with 10 mL of distilled water and treated with ferric chloride ($FeCl_3$) reagent. The formation of a blackish-green color indicated a positive result.

Terpenoid/Steroid Test

A total of 1 mL of extract solution was treated with Liebermann–Burchard reagent. The formation of a green precipitate indicated the presence of steroids, while a red precipitate indicated the presence of triterpenoids.

Saponin Test

A total of 0.5 mL of extract was added to 10 mL of hot distilled water and shaken vertically for 10 seconds. The formation of stable foam with a height of 1–10 cm persisting for 10 minutes and

remaining after the addition of one drop of 2 N hydrochloric acid indicated a positive result for saponins.

S6. Thin Layer Chromatography (TLC)

Thin layer chromatography was performed using silica gel GF₂₅₄ plates. Extract samples were prepared at a concentration of 5% (w/v) and applied to the plates. After development, the plates were sprayed with Dragendorff reagent, citroborate reagent, Liebermann–Burchard reagent, and ferric chloride (FeCl₃) to detect alkaloids, flavonoids, terpenoids/steroids, and phenolic compounds, respectively.

S7. Sterilization of Equipment and Media

Glassware and heat-resistant equipment were sterilized using dry heat in an oven at 180°C for 2 hours. Inoculation loops and forceps were sterilized by flaming. Heat-sensitive materials and culture media were sterilized using moist heat in an autoclave at 121°C for 15 minutes.

S8. Preparation of Culture Media

Brain Heart Infusion Broth (BHIB)

A total of 37 g (or 74 g for double-strength BHIB) was dissolved in 1 L of distilled water and sterilized by autoclaving at 121°C for 15 minutes.

Mueller–Hinton Agar (MHA)

A total of 38 g of MHA powder was dissolved in 1 L of distilled water by heating with constant stirring until fully dissolved, followed by sterilization at 121°C for 15 minutes.

S9. Checkerboard Microdilution Assay Layout

The checkerboard microdilution assay was performed using sterile 96-well microplates. Serial two-fold dilutions of kersen leaf extract (KLE) and beluntas leaf extract (BLE) were prepared horizontally and vertically across the plate, respectively, to obtain all possible concentration combinations. Control wells included solvent control, bacterial growth control, and media sterility control. The final volume in each well was 200 µL, consisting of extract combinations, bacterial suspension (final concentration 1 × 10⁶ CFU/mL), and BHIB medium. Plates were incubated at 37°C for 18–24 hours prior to visual assessment of bacterial growth.

S10. Determination of MIC and MBC

The minimum inhibitory concentration (MIC) was determined visually as the lowest concentration showing no visible turbidity in the broth medium. Wells corresponding to MIC values were subcultured onto Mueller–Hinton Agar plates and incubated at 37°C for 18–24 hours. The minimum bactericidal concentration (MBC) was defined as the lowest concentration showing no bacterial growth on agar plates.