

ABSTRACT

COMPARISON OF THE CHARACTERISTICS, ANTIOXIDANT ACTIVITY, and ANTIBACTERIAL ACTIVITY OF COCOA POD HUSK (*Theobroma cacao* L.) EXTRACT Against *Klebsiella pneumoniae* USING MACERATION and ULTRASOUND-ASSISTED EXTRACTION METHODS.

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Background Indonesia, as one of the major cocoa producers, generates approximately $\pm 75\%$ cocoa pod husk waste, which may cause environmental problems. Cocoa pod husk (*Theobroma cacao* L.) is rich in phytochemicals with potential antioxidant and antibacterial activities against *Klebsiella pneumoniae*, a Gram-negative ESBL-producing bacterium resistant to antibiotics. Differences in extraction methods may affect the chemical composition and biological activities of these compounds.

Methods: Cocoa pod husk from Adiluwih District, Pringsewu Regency, was extracted using 96% ethanol by maceration (1:10 for 24 h) and Ultrasound-Assisted Extraction (1:10 at 50 °C for 30 min), each performed in triplicate. The extracts were characterized by GC-MS. Total phenolic and flavonoid contents were determined using the Folin–Ciocalteu and AlCl_3 methods, respectively. Antioxidant activity was evaluated using the DPPH assay (IC_{50}), while antibacterial activity was assessed by the well diffusion method against *Klebsiella pneumoniae*.

Results: Maceration produced 58 compounds, more than Ultrasound-Assisted Extraction with 26 compounds. The total phenolic and flavonoid contents of maceration extracts were 13,20 mg GAE/g and 70,855 mg QE/g, higher than those of Ultrasound-Assisted Extraction (5,60 mg GAE/g and 54,116 mg QE/g). The IC_{50} value of maceration was 476,681 $\mu\text{g/mL}$, lower than that of Ultrasound-Assisted Extraction at 665,133 $\mu\text{g/mL}$. The maximum inhibition zone of Ultrasound-Assisted Extraction was $1,55 \pm 0,15$ mm at 30% concentration, while maceration showed $0,75 \pm 0,327$ mm at 100%.

Conclusion: Extraction methods influenced compound profiles and phenolic and flavonoid contents but did not show significant differences in antioxidant and antibacterial activities.

Keywords: Antibacterial; antioxidant; cocoa pod husk; extraction methods; *Klebsiella pneumoniae*.

ABSTRAK

PERBANDINGAN KARAKTERISTIK, AKTIVITAS ANTIOKSIDAN, ANTIBAKTERI EKSTRAK KULIT KAKAO (*Theobroma cacao* L.) TERHADAP *Klebsiella pneumoniae* dengan MASERASI dan *ULTRASOUND-ASSISTED EXTRACTION*

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Latar Belakang Indonesia sebagai salah satu produsen kakao utama di dunia menghasilkan limbah kulit kakao sekitar $\pm 75\%$ dari total buah yang berpotensi menimbulkan masalah lingkungan. Kulit kakao (*Theobroma cacao* L.) diketahui kaya senyawa fitokimia yang berpotensi sebagai antioksidan dan antibakteri terhadap *Klebsiella pneumoniae*, bakteri Gram negatif penghasil ESBL (*Extended-Spectrum Beta-Lactamase*) yang resisten terhadap antibiotik. Perbedaan metode ekstraksi dapat memengaruhi kandungan dan aktivitas biologis senyawa tersebut.

Metode: Kulit kakao dari Kecamatan Adiluwih, Kabupaten Pringsewu diekstraksi menggunakan etanol 96% melalui metode maserasi (perbandingan 1:10 selama 24 jam) dan Ultrasound-Assisted Extraction (perbandingan 1:10 pada suhu 50 °C selama 30 menit). Ekstrak dikarakterisasi menggunakan GC-MS. Total fenolik dan flavonoid ditetapkan masing-masing dengan metode Folin–Ciocalteu dan AlCl_3 . Aktivitas antioksidan diuji menggunakan metode DPPH (IC_{50}) dan aktivitas antibakteri diuji dengan metode difusi sumuran terhadap *Klebsiella pneumoniae*.

Hasil: Maserasi menghasilkan 58 senyawa, lebih banyak dibandingkan *Ultrasound-Assisted Extraction* sebanyak 26 senyawa. Total fenolik dan flavonoid maserasi masing-masing sebesar 13,20 mg GAE/g dan 70,855 mg QE/g, lebih tinggi dibandingkan *Ultrasound-Assisted Extraction* (5,60 mg GAE/g dan 54,116 mg QE/g). Nilai IC_{50} maserasi sebesar 476,681 $\mu\text{g/mL}$ lebih rendah daripada *Ultrasound-Assisted Extraction* 665,133 $\mu\text{g/mL}$. Diameter zona hambat maksimum *Ultrasound-Assisted Extraction* sebesar $1,55 \pm 0,15$ mm pada konsentrasi 30% dan maserasi $0,75 \pm 0,327$ mm pada konsentrasi 100%.

Simpulan: Metode ekstraksi memengaruhi profil senyawa serta kadar fenolik dan flavonoid, tetapi tidak menunjukkan perbedaan signifikan terhadap aktivitas antioksidan dan antibakteri.

Kata Kunci: Antibakteri; antioksidan; *Klebsiella pneumoniae*; kulit kakao; metode ekstraksi