Exploration studies of endophytic bacteria from *Arcangelisia flava* (L.) and their potential have not much been conducted. This research aims to explore and characterize the antimicrobial activity of endophytic bacteria in *A. flava* against pathogenic bacteria. This research consists of several steps including the isolation of bacteria, screening of the antimicrobial activity assay using the dual cross streak method, molecular identification through 16s rDNA analysis, and characterization of bioactive compound production through PKS-NRPS gene detection and GC-MS analysis. There are 29 endophytic bacteria that were successfully isolated from *A. flava*. The antimicrobial activity showed that there are four potential isolates AKEBG21, AKEBG23, AKEBG25, and AKEBG28 that can inhibit the growth of pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The 16s rDNA sequence analysis showed that these isolates are identified as *Bacillus cereus*. These four isolates are identified as able to produce the bioactive compounds through the detection of polyketide synthase (PKS) and nonribosomal peptide synthase (NRPS)-encoding genes. *B. cereus* AKEBG23 has the highest inhibition against pathogenic bacteria, and according to the GC-MS analysis, five major compounds are allegedly involved in its antimicrobial activity such as butylated hydroxytoluene (BHT), dioctyl phthalate, E-15-heptadecenal, 1-heneicosanol, and E-14-hexadecenal. This result suggested that *B. cereus* AKEBG23 as the endophytic bacterium from *A. flava* has a beneficial role as well as the plant itself. The bacterium produces several bioactive compounds that are allegedly involved in its antimicrobial activity against pathogenic bacteria.
1. Introduction

Enggano Island is one of the outer islands in Indonesia which administratively belongs to the Bengkulu Province (Figure 1). As one of the outer islands, Enggano has a high floral diversity in several ecosystems such as mangrove, beach, riparian, natural forest, and swamp ecosystems. The high floral diversity in Enggano included the medicinal plants utilized by local people as the traditional medicine. The local people named “kayu kuning" (yellow wood) or “akar kuning" (yellow root), which generally consists of three different species such as Arcangelisia flava (L.), Fibraurea tinctoria and Coscinium fenestratum [2]. A. flava is widely distributed in Indonesia, such as in Sumatera, Kalimantan, Sulawesi, Java, and Maluku [3].

The local people use the A. flava plants to treat several diseases such as malaria, dysentery, and fever [4, 5]. Previous research has also shown the potency of A. flava as the producer of an antioxidant, antidiabetic, and antimicrobial compound such as berberine [6–8]. Previous research also reported that the alkaloid extract from A. flava has the potential to treat skin-related fungal infections caused by Candida albicans and Trichophyton mentagrophytes [6].

The potential of A. flava as a medicinal plant is not only found in the direct usage of the plant metabolite, but also its endophytic microbes reside in it. Several endophytic microbes isolated from A. flava leaves produce the antimicrobial compound. Previous research reported that coelomycetes AFKR-18, the endophytic fungi isolated from A. flava, produce pachybasin as the major antimicrobial and anti-fungal compound against Escherichia coli, Bacillus subtilis, Micrococcus luteus, Candida albicans, and Aspergillus flavus [9]. Coelomycetes also produce phloroglucinol which has strong antimicrobial activity against E. coli [10]. Instead of fungi, a recent study also reported that some endophytic bacteria from A. flava have antimicrobial activity against E. coli [10].

According to these research works, the endophytic microbe of A. flava has the potential as the antimicrobial compound producer. The data of endophytic microbes from A. flava from Enggano Island are not well studied, especially related to the beneficial compound produced by the microbes. Therefore, the objectives of this research are to characterize the endophytic bacteria isolated from A. flava originated from Enggano Island as the potential antimicrobe and conduct the profiling of the bioactive compound produced by the bacteria.

2. Materials and Methods

2.1. Sample Collection. The A. flava samples were collected from Enggano Island (Figure 1). The plant was collected and divided into 3 parts that are roots, stems, and leaves, and each sample was stored and will be used as a source for microbial isolation.

2.2. Isolation and Purification of Bacteria. Isolation of endophytic bacteria was conducted using the serial dilution method from $10^{-1}$ to $10^{-8}$. The plant tissue was surface-sterilized, crushed, and diluted in a sterile saline solution. As much as 0.1 mL of each dilution was inoculated to nutrient agar (NA) medium using the spread plate method and incubated at 30°C for 24–48 hr. The grown colony was then gradually purified by inoculating to the new NA medium.

2.3. Antimicrobial Assay. Screening of potential antimicrobial isolates was performed using the cross streak method [12] with a few modifications against four pathogenic microbes, namely, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. The isolate was cultured overnight in tryptic soy broth (TSB) and adjusted using the McFarland turbidity standard of 0.5 so that the cells reach 1.5×10^8 CFU. As much as 1% (v/v) of pathogenic microbes were inoculated into 100 mL of tryptic soy agar (TSA) at ±40°C, homogenated, and poured onto a sterile Petri dish. The endophytic bacteria were then streaked in the TSA medium containing pathogenic microbes and incubated at 30°C for 48 hr. The clear zone around the potential endophytic bacteria indicates the antimicrobial activity. The diameter of the zone was measured to determine the strength of the antimicrobial nature of each isolate. The further antimicrobial assay was performed for the selected potential isolates with a similar method with three replicates. The assay was carried out by using a pure bacterial colony, filtrate of the medium, cell pellets, and ethyl acetate extract. As for the filtrate and ethyl acetate extract, the antimicrobial assay used was the disk diffusion method [13, 14].

2.4. Identification of Bacteria. The sequencing of 16S rDNA was used to identify the endophytic bacteria. Several selected isolates were grown in NA for 48 h for DNA extraction. The DNA was extracted using a Geneaid Presto bacterial DNA Extraction Kit (Geneaid Biotech Ltd.; New Taipei City, TW) according to the standard protocol provided by the manufacturer. Pure genomic DNA was then amplified for 16S...
rDNA using universal primer 63F/1387R [15] using the T100 Thermal Cycler (Bio-Rad Laboratories; Hercules, CA, USA). The PCR product was then sequenced using the Sanger sequencing method outsourced in a service laboratory. The 16S rDNA sequences were analyzed using ChromasPro software (Version 1.6.3; Technelysium Pte Ltd.; South Brisbane, QLD, AU) for the quality checking and trimming process. The assembled forward and reverse 16S rDNA sequences were compared to an online database in GeneBank through the basic local alignment tool for nucleotide (BLAST-N) [16]. The neighbor-joining tree was generated using MEGA version 7.0 [17] using the bootstrap method, with 1000x replicates.

2.5. Extraction and Profiling of the Bioactive Compound. Profiling of bioactive compounds was conducted through ethyl acetate extraction and then continued in gas chromatography-mass spectrophotometry (GC-MS) analysis. As much as 10 mL of 24 h potential bacterial isolates were inoculated into 1 L of the TSB medium, the suspension was incubated in a shaker incubator at 30°C for 72 h. The extraction of the metabolite from the potential isolates was conducted by the addition of ethyl acetate 1:1 (v/v) and shaking for 20 min. The upper layer of the suspension (solvent layer) was then separated and evaporated in a rotary evaporator at 42°C. The evaporated extract obtained then weighted and diluted in 1 mL dimethyl sulfoxide 50% (DMSO) to determine the concentration. GC-MS analysis was performed using Shimadzu GC-MS-QP2010 (Shimadzu Corporation; Tokyo, JPN) with RTX-5MS column. The result of mass spectra was then compared to the National Institute of Standards and Technology (NIST) database version 11 (NIST; Gaithersburg, MD, USA).

2.6. Amplification of PKS and NRPS-Encoding Gene. Amplification of polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS)-encoding genes was performed on the selected potential isolates with the highest antimicrobial activity. The PKS-encoding gene was amplified using a specific primer for the ketosynthase (KS) domain, deg.KS2F.gc 5′- GCSATGGAYCCSCARCARCGSVT-3′ and the reverse primer, deg.KSR5.gc 5′- GTSCCSSGTSCCRTGSSCYTCSAC-3′ while NRPS-encoding gene was amplified using domain NRPS specific primer forward deg.NRPS-1F.i 5′- AARDSIGGIGSIGSTAYBICC-3 and reverse deg.NRPS-4R.i 5′- CKRWAIIC- CICKIAIYTIIAYYTG-3′ [18]. PCR amplification was conducted using the T100 Thermal Cycler with the following PCR mix, 1 μL of each primer (10 μM), 1 μL LA Taq polymerase (Takara; Takara Bio; Mountain View, CA, USA), 25 μL 2X buffer with GC, 3 μL of pure genomic DNA, and nuclease-free water up to 50 μL. The PCR condition for those two-primer pairs was identical. As many as 30 PCR cycles consist of the following steps: initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 2 minutes, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and final elongation at 72°C for 10 minutes.

3. Results and Discussion

3.1. Isolation and Screening of Potential Endophytic Bacteria. There are 29 endophytic bacterial isolates successfully isolated from the roots, leaves, and stem of A. flava through the serial dilution method. These isolates have different morphological characteristics as mentioned in Table 1. 29 isolates were then roughly screened for antimicrobial activity using the cross-streak method against S. aureus, E. coli, P. aeruginosa, and C. albicans. The potential antimicrobial isolates show a clear zone around the colony (Table 1).

According to the screening results, four potential isolates qualitatively inhibited the growth of S. aureus and E. coli, namely, AKEBG21, AKEBG23, AKEBG25, and AKEBG28. These all isolates are obtained from the stem of A. flava and have similar morphological characteristics such as pale-colored colony, rod-shaped, and Gram-positive (Figure 2). These isolates will be used for the further antimicrobial assay.

3.2. Antimicrobial Activity of Selected Endophytic Bacteria. Four selected isolates were tested for further antimicrobial activity against E. coli, P. aeruginosa, S. aureus, and C. albicans. The isolates were grown in TSB overnight and centrifuged to concentrate the cells and separate the cells and its medium. The assay was conducted for the pure isolates, pelleted cells, supernatant (medium), and ethyl acetate extract for the isolates. According to the assay, all isolates can inhibit the growth of E. coli and S. aureus either in the form of pure isolates, pelleted cells, supernatant (medium), and ethyl acetate extract. However, only the ethyl acetate extract of all isolates can inhibit P. aeruginosa with relatively higher activity than filtrates and pelleted cells (Table 2).

The isolate AKEBG23 has the highest and most stable antimicrobial activity among the four isolates against the pathogenic bacteria tested. Generally, the antimicrobial activity was found higher in pure isolates and ethyl acetate extract than in pelleted cells and supernatant. Previous research reported that the cell-free supernatant from bacterial isolates mostly had no antimicrobial activity against S. aureus and B. subtilis. However, the crude extract of those isolates had clear antimicrobial activity [19]. Conversely, Danilova et al. [20] reported that the supernatant of Lactobacillus plantarum had antimicrobial activity against food spoilage and pathogenic bacteria. It is allegedly due to the two kinds of antimicrobial compounds produced extracellularly by the bacteria. According to these former research works, it can be known that the antimicrobial compound can be found either in the cell-free supernatant or in the crude extract of the bacteria.

3.3. Identification of Selected Potential Isolates. The four potential isolates obtained were identified through the sequencing of 16S rDNA. The 16S rDNA sequences are compared with the gene bank online database using BLAST-N [16] with a search set restricted to the type material so that the DNA sequences used for identification are well curated and validly identified. According to the phylogenetic tree
analysis (Figure 3), all isolates are closely related to Bacillus cereus. It is also supported by the morphological characteristics (Table 1), which mentioned that isolates AKEBG21, AKEBG23, AKEBG25, and AKEBG28 are rod-shaped, Gram-positive bacteria which are the main morphological characteristics of Bacillus.

3.4. Amplification of PKS and NRPS-Encoding Gene. Apart from the identification, detection of polyketide synthase (PKS) and nonribosomal peptide synthase (NRPS)-encoding genes was also conducted to determine the potential of each isolate to produce the bioactive compound. The detection of PKS and NRPS-encoding genes was carried out using the specific primer pair degKS2F.gc/degKSR5.gc for PKS which produce 700 bp amplicon and degNRPS-1F.i/degNRPS-4R.i for NRPS which produces 1000 bp amplicon [18].

According to the amplification, the four selected isolates have PKS and NRPS genes which indicate the potential to produce the bioactive compound. The sequence analysis

---

Table 1: Morphological characteristics and screening of endophytic bacterial isolates obtained from A. flava (L.).

| Sources | Isolate code | Gram staining | Cell shape | Inhibition activity |
|---------|--------------|---------------|------------|---------------------|
| Leave   | AKEDT4       | Positive      | Rod shape  | −                   |
|         | AKEDT5       | Negative      | Rod shape  | −                   |
|         | AKEDT10      | Positive      | Rod shape  | −                   |
|         | AKEDT12      | Positive      | Round shape| −                   |
|         | AKEBT1       | Positive      | Rod shape  | −                   |
|         | AKEBT2       | Positive      | Rod shape  | −                   |
|         | AKEBT13      | Positive      | Round shape| −                   |
|         | AKEBT14      | Positive      | Round shape| −                   |
|         | AKEBT15      | Positive      | Round shape| −                   |
|         | AKEBT16      | Positive      | Round shape| −                   |
|         | AKEBT17      | Positive      | Round shape| −                   |
|         | AKEBT18      | Positive      | Round shape| −                   |
|         | AKEBG19      | Positive      | Rod shape  | −                   |
|         | AKEBG20      | Positive      | Rod shape  | −                   |
|         | AKEBG21      | Positive      | Rod shape  | +                   |
|         | AKEBG22      | Positive      | Rod shape  | −                   |
|         | AKEBG23      | Positive      | Rod shape  | +                   |
|         | AKEBG24      | Positive      | Rod shape  | −                   |
|         | AKEBG25      | Positive      | Rod shape  | +                   |
|         | AKEBG26      | Positive      | Rod shape  | −                   |
|         | AKEBG27      | Positive      | Rod shape  | −                   |
|         | AKEBG28      | Positive      | Rod shape  | +                   |
|         | AKEBG29      | Positive      | Rod shape  | −                   |
| Stem    | AKEBT1       | Positive      | Rod shape  | −                   |
|         | AKEBT2       | Positive      | Rod shape  | −                   |
|         | AKEBT13      | Positive      | Round shape| −                   |
|         | AKEBT14      | Positive      | Round shape| −                   |
|         | AKEBT15      | Positive      | Round shape| −                   |
|         | AKEBT16      | Positive      | Round shape| −                   |
|         | AKEBT17      | Positive      | Round shape| −                   |
|         | AKEBT18      | Positive      | Round shape| −                   |
|         | AKEBG19      | Positive      | Rod shape  | −                   |
|         | AKEBG20      | Positive      | Rod shape  | −                   |
|         | AKEBG21      | Positive      | Rod shape  | −                   |
|         | AKEBG22      | Positive      | Rod shape  | −                   |
|         | AKEBG23      | Positive      | Rod shape  | −                   |
|         | AKEBG24      | Positive      | Rod shape  | −                   |
|         | AKEBG25      | Positive      | Rod shape  | −                   |
|         | AKEBG26      | Positive      | Rod shape  | −                   |
|         | AKEBG27      | Positive      | Rod shape  | −                   |
|         | AKEBG28      | Positive      | Rod shape  | −                   |
|         | AKEBG29      | Positive      | Rod shape  | −                   |
| Root    | AKEAT3       | Positive      | Rod shape  | −                   |
|         | AKEAT6       | Positive      | Rod shape  | −                   |
|         | AKEAT7       | Positive      | Rod shape  | −                   |
|         | AKEAT8       | Positive      | Rod shape  | −                   |
|         | AKEAT9       | Positive      | Rod shape  | −                   |
Table 2: The antimicrobial assay of four selected endophytic isolates.

| Sources                     | Isolate codes | Inhibition zone diameter (mm) | E. coli | S. aureus | P. aeruginosa | C. albicans |
|-----------------------------|---------------|-------------------------------|---------|-----------|---------------|-------------|
|                             |               |                               |         |           |               |             |
|                             | Pure isolates (first screening) |                               |         |           |               |             |
| AKEBG21                     | 2.70 ± 0.23   | 12.7 ± 1.19                  | 0.00 ± 0.00 | 0.00 ± 0.00 |
| AKEBG23                     | 22.20 ± 1.20  | 23.8 ± 0.70                  | 0.00 ± 0.00 | 0.00 ± 0.00 |
| AKEBG25                     | 12.70 ± 0.98  | 19.5 ± 0.45                  | 0.00 ± 0.00 | 0.00 ± 0.00 |
| AKEBG28                     | 15.80 ± 1.75  | 16.7 ± 0.11                  | 0.00 ± 0.00 | 0.00 ± 0.00 |
| PC+*                       | 20.80 ± 0.22  | 22.40 ± 0.03                 | 18.30 ± 0.07 | 25.08 ± 0.02 |
| NC−*                       | 0.00 ± 0.00   | 0.00 ± 0.00                  | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                             | AKEBG21       | 2.10 ± 0.56                  | 1.40 ± 0.56 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                             | AKEBG23       | 4.00 ± 0.70                  | 1.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                             | AKEBG25       | 1.10 ± 0.07                  | 0.50 ± 0.28 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                             | AKEBG28       | 3.10 ± 0.07                  | 0.70 ± 0.28 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                             | PC+*          | 23.60 ± 0.45                 | 19.50 ± 0.54 | 18.34 ± 0.23 | 20.00 ± 0.01 |
|                             | NC−*          | 0.00 ± 0.00                  | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                             | Medium supernatant |                               |         |           |               |             |
| AKEBG21                     | 19.10 ± 0.84  | 13.85 ± 0.49                 | 3.15 ± 1.82 | 0.00 ± 0.00 |
| AKEBG23                     | 20.85 ± 0.07  | 23.15 ± 1.34                 | 22.65 ± 0.21 | 0.00 ± 0.00 |
| AKEBG25                     | 19.80 ± 0.50  | 14.7 ± 0.28                  | 11.65 ± 0.63 | 0.00 ± 0.00 |
| AKEBG28                     | 21.50 ± 0.42  | 14.6 ± 0.00                  | 13.35 ± 0.63 | 0.00 ± 0.00 |
|                             | PC+*          | 16.40 ± 0.28                 | 19.5 ± 0.00 | 18.25 ± 0.63 | 16.0 ± 0.00 |
|                             | NC−*          | 0.00 ± 0.00                  | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
|                             | Crude extract (ethyl acetate) |                               |         |           |               |             |
| AKEBG21                     | 19.10 ± 0.84  | 13.85 ± 0.49                 | 3.15 ± 1.82 | 0.00 ± 0.00 |
| AKEBG23                     | 20.85 ± 0.07  | 23.15 ± 1.34                 | 22.65 ± 0.21 | 0.00 ± 0.00 |
| AKEBG25                     | 19.80 ± 0.50  | 14.7 ± 0.28                  | 11.65 ± 0.63 | 0.00 ± 0.00 |
| AKEBG28                     | 21.50 ± 0.42  | 14.6 ± 0.00                  | 13.35 ± 0.63 | 0.00 ± 0.00 |
|                             | PC+*          | 16.40 ± 0.28                 | 19.5 ± 0.00 | 18.25 ± 0.63 | 16.0 ± 0.00 |
|                             | NC−*          | 0.00 ± 0.00                  | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |

Positive control: streptomycin 1000 ppm for bacteria (S. aureus, E. coli, and P. aeruginosa); kanamycin 1000 ppm for C. albicans. Negative control: NC−*: sterile TSB medium and NC−**: ethyl acetate.

Figure 3: Neighbor-joining tree of four potential bacterial isolates with the highest antimicrobial activity. The tree was tested using the bootstrap method with 1000x replicates.
Table 3: BLAST-X result of KS-domain amplified from four selected endophytic isolates.

| Isolate code | Acc. no.            | Description                                                                 | Similarity (%) | E-value |
|--------------|---------------------|-------------------------------------------------------------------------------|----------------|---------|
| AKEBG21      | VAM49526.1          | 6-deoxyerythronolide-B synthase, Glutamate-1-semialdehyde 2,1-aminomutase    | 99.46          | 7e-117  |
|              | WP_203462309.1      | (Enterobacter cloacae) Aminotransferase class III-fold pyridoxal phosphate-dependent enzyme | 99.46          | 7e-117  |
| AKEBG23      | SAJ35050.1          | Ketosynthase (Bacillus subtilis)                                             | 95.87          | 2e-152  |
|              | WP_032727815.1      | Type I polyketide synthase (Bacillus subtilis)                              | 95.41          | 7e-148  |
| AKEBG25      | WP_032727815.1      | Type I polyketide synthase (Bacillus subtilis)                              | 98.97          | 8e-137  |
|              | WP_187956203.1      | Polyketide synthase PksL (Bacillus subtilis)                                 | 98.97          | 5e-128  |
|              | SAJ35050.1          | Ketosynthase (Bacillus subtilis)                                             | 99.53          | 2e-159  |
| AKEBG28      | WP_032727815.1      | Type I polyketide synthase (Bacillus subtilis)                              | 99.07          | 8e-155  |
through BLAST-X B. cereus AKEBG21 PKS gene is closely related to 6-deoxyerythronolide-B synthase, glutamate-1-semialdehyde 2,1-aminomutase from Enterobacter cloacae, and B. cereus AKEBG23, AKEBG25, and AKEBG28 PKS genes are close with Bacillus subtilis ketosynthase type I (Table 3). 6-deoxyerythronolide-B synthase and glutamate-1-semialdehyde 2,1-aminomutase from B. cereus AKEBG21 were detected as closely related enzymes from the KS-domain of AKE. According to the conserved domain database, it shares the similar PksD domain with polyketide synthase (PKS) under the superfamily condensing enzyme and decarboxylating condensing enzyme family [21].

The NRPS gene analysis shows that all amplified NRPS fragments were closely related to the Bacillus NRPS gene with various similarities (Table 4). Previous research has showed that Bacillus subtilis and Bacillus flexus with antimicrobial activity against Vibrio species were also harboring PKS and NRPS genes [22]. Polypeptide synthase (PKS) and nonribosomal peptide synthetase are the enzymes with multidomains which are responsible for bioactive compound production in many microorganisms, specifically bacteria, actinobacteria, and fungi. Moreover, the NRPS gene has a significant role with PKS in bioactive compound biosynthesis which is composed of peptides such as polypeptides and lipopeptides [23].

3.5. Profiling of the Active Compound. As the isolate with the highest antimicrobial activity, the GC-MS profile of B. cereus AKEBG23 ethyl acetate extract shows that there are 16 bioactive compounds identified in the crude extract (Figure 4). These bioactive compounds were allegedly involved directly and indirectly in the antimicrobial activity of B. cereus AKEBG23 against four pathogenic bacteria. There are five major compounds associated with antimicrobial activity, namely, butylated hydroxytoluene, diisooctyl phthalate, E-15-heptadecenal, 1-heneicosanol, and E-14-hexadecenal (Table 5).

Butylated hydroxytoluene (BHT) is known as an antioxidant, which is commonly used as a standard antioxidant [51]. This compound was found as the highest detected compound in GC-MS analysis. Instead of controlling the oxidation process, the bioactivity of BHT is also reported as antimicrobial against Staphylococcus aureus [52]. Another research also reported that BHT was also found in the extract of green algae Scenedesmus obliquus with antimicrobial bioactivity [31].

The second highest detected compound is diisooctyl phthalate which is mostly known as human-made pollutant phthalic acid esters (PAE). However, several research studies reported that PAE is produced by plants and microorganisms. PAE is also reported to have allelopathic, antimicrobial, and insecticidal activities which increase the competitiveness of the producer [53]. Diisooctyl phthalate is one of the PAE produced by fungi Fusarium oxysporum and Phoma herbarum and microalgae Nostoc sp.; it also has the antimicrobial activity against Staphylococcus aureus, Klebsiella pneumoniae, Trichophyton mentagrophytes, and Candida albicans [50, 54]. The reports related to diisooctyl phthalate produced by bacteria are limited; this can be a new report that finds diisooctyl phthalate in bacteria, especially B. cereus.

Another major compound found in B. cereus AKEBG23 ethyl acetate extract is E-15-heptadecenal, 1-heneicosanol, and E-14-hexadecenal. E-15-heptadecenal and E-14-hexadecenal are reported to be involved in antifungal activity. E-15-heptadecenal is found in the B. siamensis active compound profile which is allegedly involved in antimicrobial resistance.
Table 5: Bioactive compound profile of *B. cereus* AKEBG23.

| No | Retention time | % Area | Name                                           | Chemical formula | Similarity (%) | Bioactivity                                                                 |
|----|----------------|--------|-----------------------------------------------|------------------|----------------|-----------------------------------------------------------------------------|
| 1  | 7.908          | 0.39   | 1-Dodecanol                                   | C₁₂H₂₆O          | 95             | Antibacterial activity [24]                                               |
| 2  | 11.027         | 2.20   | E-14-hexadecenal                              | C₁₆H₃₀O          | 95             | Antifungi [25]                                                             |
| 3  | 11.172         | 0.48   | Tetradecane                                   | C₁₄H₃₀           | 95             | Antioxidant, antimicrobial [26], and anti-inflammatory [27]                |
| 4  | 12.951         | 0.78   | 2-Propenoic acid, tetraetyl ester             | C₁₅H₂₄O₂         | 75             | Antioxidant activity [28]                                                 |
| 5  | 13.986         | 57.13  | Butylated hydroxytoluene                      | C₁₅H₂₄O          | 95             | Antioxidant [29, 30]; antimicrobe [31]                                     |
| 6  | 15.637         | 3.55   | E-15-heptadecenal                             | C₁₅H₂₄O          | 95             | Antimicrobe [32, 33]                                                       |
| 7  | 18.034         | 3.04   | 2-Propenoic acid, tetraetyl ester             | C₁₇H₃₂O₂         | 88             | —                                                                           |
| 8  | 18.716         | 0.87   | Spiro-1-(cyclohex-2-ene)-2’-(5’-oxabicyclo(2.1.0)pentane), 1’4,2,6,6-pentamethyl-| C₁₄H₂₂O          | 76             | Biomarkers to detect the presence of toxigenic fungal pathogen [34]         |
| 9  | 19.574         | 0.58   | Phenol, 4-(1,1,3,3-tetramethylbutyl)-         | C₁₄H₂₂O          | 92             | —                                                                           |
| 10 | 20.220         | 3.06   | E-15-heptadecenal                             | C₁₅H₂₄O          | 95             | Antimicrobe [35]                                                           |
| 11 | 23.083         | 2.57   | Pyrrolo (1,2-a) pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-l-(+)-Ascorbic acid 2,6-dihexadecanoate | C₁₃H₁₈N₂O₂      | 90             | Antioxidant [36]                                                           |
| 12 | 23.885         | 5.06   | 1-Heneicosanol                                | C₂₂H₄₄O₈         | 86             | Antimicrobial and antitumor [37, 38]                                       |
| 13 | 24.377         | 2.26   | n-Tetracosanol-1                              | C₂₄H₅₀O          | 94             | Antimicrobial and antifungi [39]; Antimycobacteria [40, 41]                |
| 14 | 28.133         | 1.00   | 1-Heptacosanol                                | C₂₄H₃₈O₄         | 93             | Antibacterial [42]; anti-inflammatory [43]; antioxidant [44]               |
| 15 | 31.556         | 0.42   | 1-Heptacosanol                                | C₂₄H₃₂O₄         | 96             | Antibacterial [45]; antifungal [46]                                       |
| 16 | 34.145         | 16.61  | *Diisooctyl phthalate*                        | C₂₄H₃₂O₄         | 96             | Antimicrobe [47–49]; antitumor [50]                                        |
activity of B. siamensis C.38 against some phytopathogenic fungi [32], while E-14-hexadecenal is the antifungal bioactive compound found in the endophytic fungi Pseudarthria viscida (L.) [25, 55]. 1-Heneicosanol is the long-chain fatty alcohol which is reported to have antimicrobial activity against some pathogenic bacteria. Several research studies reported that 1-heneicosanol is found in the plant Senecio coluhaapiensis and actinomycetes Streptomyces carpaticus [39, 56].

Data Availability

The GC-MS, PKS, and NRPS sequence data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

The authors Sipriyadi and Masrulkin equally contributed to this work.

Acknowledgments

This study was supported by the Research and Community Services Centre (LPPM), the University of Bengkulu through 2019 Research Grant number 2338/UN30.14/HK/2020 dated 24 August, 2020. The authors acknowledge all parties who have helped the completion of this study. The authors are also grateful to the people of Enggano Island, Bengkulu Province, and the University of Bengkulu through 2019 Research Grant number:2338/UN30.14/HK/2020 dated 24 August, 2020. The authors are also grateful to the people of Enggano Island, Bengkulu Province, and all parties who have helped the completion of this research.

References

[1] D. Novira, Lismaningsih, and T. Bungsu, “The exploration of local knowledge on ethnomedicine in traditional healing practices at Enggano Island Bengkulu Province,” Research on Humanities and Social Sciences, vol. 10, pp. 95–102, 2020.
[2] R. Sulistiarini, A. A. Soemardji, Elfahmi, and M. I. Iwo, “Pharmacological activities of three kinds "kayu kuning": Arcangelisia flava, Fibrarea tinctoria and Coscinium fenestratum-a short review,” Journal of Tropical Pharmacy and Chemistry, vol. 5, no. 4, pp. 381–386, 2021.
[3] GBIF Secretariat, “GBIF backbone taxonomy,” 2021, https://www.gbif.org/species/3830318.
[4] H. Subeki, H. Matsuura, K. Takahashi et al., “Antibacterial activity of protoberberine alkaloids and 20-hydroxyecdysone from Arcangelisia flava against Babesia gibsoni in culture,” Journal of Veterinary Medical Science, vol. 67, no. 2, pp. 223–227, 2005.
[5] E. R. Lovin, H. Arwati, and R. Rb., “In vitro intraerythrocytic antimalarial activity of akar kuning,” Journal of Folia Medica Indonesiana, vol. 48, pp. 90–95, 2012.
[6] R. Setyowati, S. Sudoarsono, and P. Setyowati, “The effect of water-soluble stem extract “kayu kuning” (Arcangelisia flava L.Merr) on the growth inhibition of Candida albicans ATCC 10231 and Trichophyton mentagrophytes in vitro,” Biology, Medicine, and Natural Product Chemistry, vol. 3, 2014.
[7] A. T. Wahyudi, J. A. Priyanto, W. Maharstwi, and R. I. Astuti, “Screening and characterization of sponge-associated bacteria producing bioactive compounds anti-Vibrio sp,” American Journal of Biochemistry and Biotechnology, vol. 14, no. 3, pp. 221–229, 2018.
[8] M. R. Fadhil Pratama, S. Suratno, and E. V. L. Mulyani, “Antibacterial activity of akar kuning (Arcangelisia flava) secondary metabolites: molecular docking approach,” Asian Journal of Pharmaceutical and Clinical Research, vol. 11, pp. 447–451, 2018.
[9] D. Wulansari, Y. Jamal, Praptiwi, and A. Agusta, “Pachybasin, a major metabolite from culture broth of endophytic Coelomycetes AFKR-18 fungus isolated from a yellow moonsheet plant, Arcangelisia flava (L.) Merr,” HAYATI Journal of Biosciences, vol. 21, no. 2, pp. 95–100, 2014.
[10] Y. Jamal, P. Praptiwi, A. Fathoni, and A. Agusta, “Bioproduksi floroglusinololeh jamur endofit Coelomycetes AFAS-E3 yang disolasi dari tumbuhan Arcangelisia flava L. Merr. Berk,” Berkala Penelitian Hayati, vol. 16, no. 2, pp. 169–172, 2011.
[11] S. R. Sipriyadi, W. Darwis, R. Hadi, R. Supriati, and S. R. Siboro, “Isolation and identification of cellulase-producing endophytic bacteria from yellow root plants (Arcangelisia flava (L.) Merr) from Enggano Island,” Proceedings 3rd KOBI Congress, International and National Conferences (KOBICINC2020), vol. 14, pp. 523–528, 2021.
[12] M. Lertcanawanichakul and S. Sawangnop, “A comparison of two methods used for measuring the antagonistic activity of Bacillus species,” Walalak Journal of Science and Technology, vol. 5, pp. 161–171, 2008.
[13] N. G. Heatley, “A method for the assay of penicillin,” Biochemical Journal, vol. 38, no. 1, pp. 61–65, 1944.
[14] M. Balouiri, M. Sadiki, and S. K. Ibnsouda, “Methods for in vitro evaluating antimicrobial activity: a review,” Journal of Pharmaceutical Analysis, vol. 6, no. 2, pp. 71–79, 2016.
[15] J. R. Marchesi, T. Sato, A. J. Weightman et al., “Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA,” Applied and Environmental Microbiology, vol. 64, no. 2, pp. 795–799, 1998.
[16] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, and D. J. Lipman, “Basic local alignment search tool,” Journal of Molecular Biology, vol. 215, no. 3, pp. 403–410, 1990.
[17] S. Kumar, G. Stecher, and K. Tamura, “MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets,” Molecular Biology and Evolution, vol. 33, no. 7, pp. 1870–1874, 2016.

International Journal of Microbiology
[21] A. Marchler-Bauer, M. K. Derbyshire, N. R. Gonzales et al., “CDD: NCBI's conserved domain database,” Nucleic Acids Research, vol. 43, no. D1, pp. D222–D226, 2015.

[22] M. Francis, M. Chacha, P. A. Ndakidemi, and E. R. Mbega, “Antifungal effects against Phaeosariopsis personata under greenhouse conditions and phytochemical analysis of Jatropha curcas leaf extracts,” International Journal of Agriculture & Material Science, vol. 2, no. 3, pp. 531–542, 2018.

[23] R. H. Hameed, G. J. Mohammed, and I. H. Hameed, “Biological activity of 1–hexenicosanol isolated from Senecio colhusiapennis, an endemic species from patagonia argentina,” Pharmaceutical Chemistry Journal, vol. 3, pp. 73–77, 2016.

[24] S. Poongulali and M. Sundararaman, “Antimycobacterial, anticanadial and antioxidant properties of Terminalia catappa and analysis of their bioactive chemicals,” International Journal of Pharmacy and Biological Sciences, vol. 6, pp. 69–83, 2016.

[25] N. Francois, N. M. Charity, O. Fred et al., “Antimycobacterial activities, cytotoxicity and phytochemical screening of extracts for three medicinal plants growing in Kenya,” Journal of Medicinal Plants Research, vol. 14, no. 4, pp. 129–143, 2020.

[26] K. Thirunavukkarasu, P. Rajkumar, S. Selvaraj, and S. Kumaressan, “GC-MS analysis of Gymnema sylvestre leaves methanolic extract for anti diabetic and anticancer drug identification,” Journal of Chemical and Pharmaceutical Sciences, vol. 9, pp. 1011–1013, 2016.

[27] S. K. Sudiriga and I. K. Ginantara, “Identification of bioactive compounds of Ficus septica leaf extract has potential as botanical pesticides to control anthracnose disease on chili pepper,” Journal of Biological Chemistry, vol. 34, pp. 150–159, 2017.

[28] M. Jalal Ali, E. A. Makky, S. Zareen, and M. M. Yusof, “Identification of bioactive phytochemicals using GC-Mass and TLC to the estimation of antimicrobial susceptibility of plant extracts,” Journal of Physics: Conference Series, vol. 1294, no. 6, pp. 62013–62110, 2019.

[29] S. F. Gheda and A. A. Ismail, “Natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities,” Anais da Academia Brasileira de Ciencias, vol. 92, no. 2, pp. 201909344–20191018, 2020.

[30] N. H. Nguyen, T. T. Nguyen, P. C. Ma, Q. T. H. Ta, T. H. Duong, and V. G. Vo, “Potential antimicrobial and anticancer activities of an ethanol extract from Bowea macrophylla,” Molecules, vol. 25, no. 8, p. 1996, 2020.
International Journal of Microbiology

[50] N. El-Zawawy, M. Metwally, and N. Abd El-Salam, "Antitumor and antimicrobial activities of endophytic fungi obtained from Egyptian urospermum picroides," International Journal of Cancer and Biomedical Research, vol. 4, pp. 187–199, 2020.

[51] A. Aras, E. Bursal, Y. Alan, F. Türkan, H. Alkan, and Ö. Kılıç, "Polyphenolic content, antioxidant potential and antimicrobial activity of satureja boissieri," Iranian Journal of Chemistry and Chemical Engineering (International English Edition), vol. 37, pp. 209–219, 2018.

[52] M. Ayaz, L. O. Luedecke, and A. L. Branen, "Antimicrobial effect of butylated hydroxyanisole and butylated hydroxytoluene on Staphylococcus aureus," Journal of Food Protection, vol. 43, no. 1, pp. 4–6, 1980.

[53] L. Huang, X. Zhu, S. Zhou et al., "Phthalic acid esters: natural sources and biological activities," Toxins, vol. 13, no. 7, pp. 495–517, 2021.

[54] O. M. A. Salem, S. M. Ghazi, and S. N. Hanna, "Antimicrobial activity of microalgal extracts with special emphasize on Nostoc sp," Life Science Journal, vol. 11, pp. 752–758, 2014.

[55] D. Abraham, B. Nair, and G. Mallikarjunaswamy, "Antimicrobial and GC-MS analysis of chloroform extract of Pseuderanthria viscida (L.) Wight and Arn. and associated major fungal endophyte," International Journal of Advanced Research, vol. 7, no. 1, pp. 105–113, 2019.

[56] R. Balachandar, N. Karmegam, M. Saravanan, R. Subbaiya, and P. Gurumoorthy, "Synthesis of bioactive compounds from vermicast isolated actinomycetes species and its antimicrobial activity against human pathogenic bacteria," Microbial Pathogenesis, vol. 121, pp. 155–165, 2018.