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Secondary Metabolites Profiling and Antimicrobial Activities of Ethanol Extract from Jamblang (*Syzygium cumini* L.) Stem Bark

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Abstract: This study was conducted to determine the secondary metabolite compounds and evaluate the antimicrobial activity of ethanol extract of jamblang stem bark (*Syzygium cumini*). The method used in this research is experimental method. The extraction was carried out using the maceration method with 96% ethanol as the solvent. Secondary metabolite compounds of the extract samples were analyzed using a Gas Chromatography-Mass Spectrometry (GC-MS). Antimicrobial activity of the extract against *Cutibacterium acnes*, *Staphylococcus aureus*, and *Candida albicans* using disc diffusion method tested on MHA (Mueller Hinton Agar) media with extract concentrations of 40%, 60%, 80%, and 100%. Each experiment was repeated three times to ensure validity. The results revealed that the extract have dominant compounds such as 7-Tetradecenal (Z), Octadecanoic acid, and n-Hexadecanoic acid. The antimicrobial test showed that the highest inhibition zones were 7.45 mm for *C. acnes*, 8.04 mm for *S. aureus*, and 4.60 mm for *C. albicans* at 100% extract concentration. The inhibition zones increased with higher extract concentrations, indicating a dose-dependent relationship. Compared to the positive control results (20.70 mm; 22.29 mm; and 18.62 mm) the extract showed moderate antimicrobial activity. These findings suggest that jamblang stem bark extract has potential as a natural antimicrobial agent.

Keywords: *Candida albicans; Cutibacterium acnes;* jamblang stem bark; secondary metabolites; *Staphylococcus aureus*.

Introduction

The prevalence of skin infection diseases has been increasing in recent decades. The global incidence rate of bacterial skin diseases increased from 8,988.74 per 100,000 in 1990 to 10,823.88 per 100,000 in 2021, with an average annual percent change (AAPC) of 0.62%. The total number of cases rose to nearly 90 million in 2021 and is projected to reach 1.2 billion by 2045 (Gu et al., 2025). Today, skin infections have become one of the worldwide causes of morbidity and mortality in the human population. All microorganisms including bacteria, viruses, parasites and fungi cause various infections that affect every organ of the body. Infections that often occur among the public are skin disease infections, which can be in the form of acne, boils, tans, and others. There are many people who choose natural medicines to treat infections and prevent side effects due to synthetic drugs (Rasnovi & Nursanty, 2015).

Cutibacterium acnes (*C. acnes*) is the main pathogenic cause of acne. In addition, *C. acnes* is often found in multispecies biofilms with skin colonizing microorganisms such as *Staphylococcus aureus* and *Candida albicans* (Lee et al., 2022). *S. aureus* bacteria can cause skin infections, food poisoning, and systemic infections, and can even cause skin infections when

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these bacteria contact wounds (Muthmainnah et al., 2016). *Candida albicans* (*C. albicans*) is the main pathogenic species that causes candidiasis. Candidiasis can affect the skin, hair, nails, mucous membrans and internal organs. Apart from being a pathogen, *C. albicans* itself is a normal flora that plays a role in the balance of microorganisms in our body (Indrayati & Sari, 2018).

Antibiotics or synthetic drugs are often used to fight these skin pathogens. However, the resistance of pathogenic bacteria and fungi to the antibiotics used causes people to believe in the medicinal properties of plants. One of them is jamblang. The jamblang plant is believed to treat various diseases, be it the fruit, leaves, seeds or stems. The benefits taken from jamblang, that is by making it an antioxidant, anti-inflammatory, deworming, anticancer, antibacterial, and antidiabetic. (Haroon et al., 2015).

The flavonoid content in jamblang can inhibit the growth of Gram-positive and Gram-negative bacteria (Sudarmi et al., 2017). Flavonoids, tannins, and essential oils contained in it can be used as antifungal. Cell wall destruction by tannins can also inhibit the growth of fungi. Fungal cell division or proliferation is inhibited by the presence of flavonoids. Essential oils will damage proteins to disrupt the activity of fungal cells until the fungal cells die (Chismirina & Rezeki, 2014). flavonoids, tannins, and essential oils employ multiple mechanisms to exert their antimicrobial effects, including disrupting cell membranes, inhibiting biofilm formation, and interfering with metabolic processes. These properties make them valuable in the fight against antibiotic-resistant bacteria and highlight their potential for therapeutic applications.

Previous studies showed that some compounds contained in jamblang bark are flavonoids, tannins, betulinic acid, friedelin, epifriedenalol, β sitosterol, eugenine, fatty acid esters, and bergenin (Ayyanar & Subash-Babu, 2012). These compounds can be potential as antimicrobial substances, therefore further research was conducted on secondary metabolite compounds contained in jamblang stem bark extract, as well as antimicrobial ability against the growth of *Cutibacterium acnes*, *Staphylococcus aureus*, and *Candida albicans* to determine the correlation between secondary metabolite compounds and antimicrobial properties of ethanol extract of jamblang stem bark.

Method

Tools and Materials

Jamblang stem bark (*Syzygium cumini*), *Cutibacterium acnes*, *Staphylococcus aureus*, and *Candida albicans* (Microbiology Laboratory, USK, Banda Aceh, Indonesia), Mc Farland standard solution (0,5), Nutrient Agar (NA), Potato Dextrose Agar (PDA), Mueller Hinton Agar (MHA) (NA, PDA, MHA medium Merck KGaA, Darmstadt, Germany), normal saline solution, distilled water, denatured alcohol, 70% alcohol (OneMed, PT Jayamas Medica Industri, Sidoarjo, Indonesia), 96% ethanol, chloramphenicole discs (Thermo Scientific Oxoid, UK) as control positive for fungi, clindamycin (Thermo Scientific Oxoid, UK) as control positive for bacteria, Laminar Air Flow (LAF), Shimadzu GC-MS-QP2010 Ultra, vortex, vernier, ose, incubator, analytical balance, petri dish, beaker glass, autoclave, test tube, micropipette, rotary evaporator, bunsen, and Erlenmeyer flask.

Preparation of Ethanol Extract of Jamblang Stem Bark

Jamblang stem bark was washed, dried, and chopped into small pieces. The dried bark was then pulverized into simplicial (fine powder) powder with a particle size of 60 mesh. Soak 1 kg of simplicia in 3 liters of 96% ethanol for 4x24 hours and then macerate it. After that, the filtrate was filtered and concentrated with a rotary evaporator at 40^{o}C. The extract is put into a vial bottle.

GC-MS Analysis of Ethanol Extract of Jamblang Stem Bark

The bioactive compounds of the extract samples were analyzed using a Gas Chromatography Mass Spectrometry (GC-MS) apparatus with a film thickness of 30 m x 0.25 mm x 0.25 µm. The injection port and temperature were set at 250°C and 280°C, respectively. A sample of 5 µL was injected with a split ratio of 8:1. The carrier gas used was Helium (He) with a flow rate of 1.2 mL/minute. The oven temperature started at 80^{o}C held for 0 minutes, increased to 150^{o}C at a rate of 3^{o}C/minute held for 1 minute and the temperature increased to 280^{o}C at a rate of 20^{o}C/minute held for 26 minutes. The ion source temperature is 230°Cand the quadrupole temperature is 140°C. While Electron Impact is 70 eV. he compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) library.

Antimicrobial Activity Test

Sterilize tools and materials using an autoclave. Prepare NA media for *C. acnes* and *S. aureus* growth, PDA media for *C. albicans* growth, and MHA media for antimicrobial testing. Isolates of each microorganism were purified on new growth media using Ose, then incubated for 24 hours. Colonies that grow are taken with Ose and then put into a test tube and mixed with normal saline solution, then the turbidity of the test microbial suspension was adjusted to match the standard 0.5 McFarland solution. Then the suspension was inoculated into MHA media using a spreader bar.

Discs that have been soaked with ethanol extract of jamblang bark with the concentration according to the treatment, then placed on MHA media that has been inoculated with microorganisms. Then incubate for 24 hours at 37 ^{o}C, after that measure the diameter of the inhibition zone formed on MHA using a caliper.

Result and Discussion

Secondary Metabolites Profiling

The secondary metabolite compounds in the extracts were tested using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. This method works by separating compounds in the mixture based on their volatility and polarity, followed by identification of compounds using mass spectra. In GC-MS analysis, compounds are detected as peaks on the chromatogram (Figure 1), each representing a specific compound. The retention time indicates the duration of the compound passing through the chromatographic column until it is detected, which is influenced by the chemical nature of the compound.

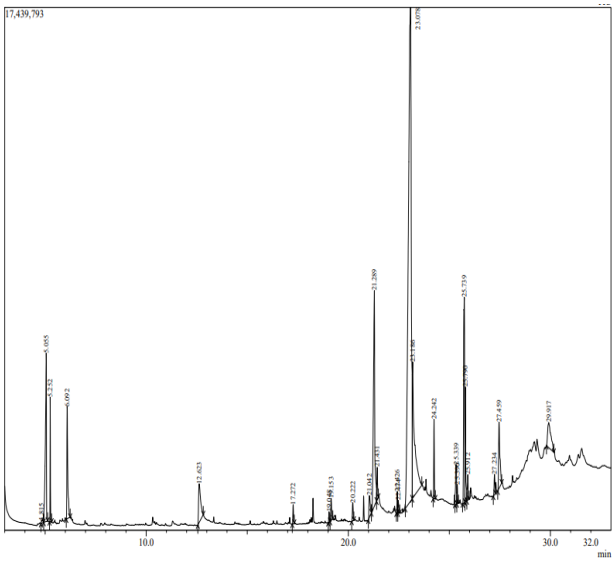

Figure 1. Chromatogram of ethanol extract of jamblang stem bark

The chromatogram based on Figure 1 shows 25 peaks indicating the presence of various secondary metabolite compounds in the ethanol extract of jamblang stem bark. The peak with the highest intensity appeared at a retention time of about 23.078, indicating that the compound was the dominant component in the extract. From the mass spectrum analysis, the compound identified with the highest relative abundance was 7-Tetradecenal (Z)-, which belongs to the Unsaturated aldehyde.

The percentage area of each peak indicates the relative contribution of the compound to the total extract mixture, describing its relative quantity in the sample. Different retention times on the chromatogram indicate the presence of components with diverse polarity characteristics (Handayani et al., 2020). Meanwhile, the similarity index is generated from matching the mass spectrum of the compound with the available spectrum library (database), with values close to 100% indicating a high level of accuracy in compound identification). GC-MS analysis results of ethanol extract of jamblang stem bark showed there were twenty-five peaks (Table 1).

Table 1. GC-MS results of ethanol extract of jamblang stem bark

Peak	Compound name	RT	Area (%)	SI	Category
1	Formic acid	4.82	0.24	96	Carboxylic acid
2	Acetic acid (CAS) Ethylic acid	5.05	5.65	99	Carboxylic acid
3	2-Propanone, 1-hydroxy- (CAS) Acetol	5.25	1.39	98	Alcohol and ketone
4	Furfural	6.09	3.16	98	Heterocyclic aromatics
5	Hydroxymeth furfur	12.62	2.54	91	Aldehydes
6	Diethyl Phthalate	17.27	0.36	94	Phthalate Ester
7	Alpha Hexyl Cinnamic Aldehyd	19.05	0.19	96	Aldehydes
8	Tetradecanoic acid (CAS) Myristic	19.15	0.57	97	Saturated fatty acid
9	Hexamethyl-pyranoindane	20.22	0.45	93	Polycyclic hydrocarbons
10	9-Hexadecenoic acid (CAS)	21.04	0.89	98	Unsaturated fatty acid
11	n-Hexadecanoic Acid	21.29	9.34	88	Palmitic acids
12	Hexadecanoic acid, ethyl ester	21.43	0.70	95	Palmitic acids
13	9,1 Octadecadieno acid, methyl est	22.45	0.40	96	Linoleic acids
15	7-Tetradecenal, (Z)	23.08	45.97	92	Unsaturated aldehyde
16	Octadecanoic acid	23.19	9.56	95	Stearic acids
17	Hexadecanoic acid, 2-hydroxy-1,3-propaned	24.24	1.32	85	Hydroxy fatty acid
18	Oleoyl chloride	25.34	1.35	90	Acyl chloride

Peak	Compound name	RT	Area (%)	SI	Category
19	9,12,1 Octadecatrieno acid, (Z,Z,Z	25.39	0.33	89	Unsaturated fatty acid
20	Di-(9-Octadecenoyl)-Glycerol	25.74	6.54	88	Glycerides
21	9,12,1 Octadecatrieno acid, (Z,Z,Z	25.79	2.24	92	Unsaturated fatty acid
22	Octadecanoic acid, 2-hydroxy-1,3-propaned	25.91	0.68	84	Hydroxy fatty acid
23	cis-9-Hexadecenal	27.23	0.53	90	Aldehydes
24	Propylene Glycol Monoleate	27.46	2.66	89	Fatty acids esters
25	Eicosanoic acid, 2-[(1-oxohexadecyl) oxy]-1	29.92	2.61	77	Hydroxylated fatty acids

Notes: Retention Time (RT), Similarity Index (SI)

Based on the GC-MS analysis results in Table 1, several compounds were successfully identified in the jamblang stem bark extract. Three compounds with the largest percentage area are 7-Tetradecenal (Z) (45.97%), Octadecanoic acid (9.56%), and Hexadecanoic acid (9.34%). The three dominant compounds are unsaturated aldehyde derivatives of fatty acids (stearic acid, and palmitic acid). According to Kim (2020), in plants, fatty acids are the main lipid component that plays a role in maintaining cell integrity. According to Maphetu et al. (2022), various types of fatty acid compounds provide pharmacological effects such as antimicrobial, antioxidant, insecticide, anthelmintic, and antinociceptive.

The presence of 7-Tetradecenal (Z) as the majority component in this extract indicates its potential as one of the main bioactive compounds. 7-Tetradecenal exhibits antimicrobial activity, which may serve to inhibit bacterial growth by affecting the bacterial cell membrane. This mechanism involves the interaction between the bioactive compound and the bacterial cell wall, which may disrupt the synthesis of essential bacterial components (Ripanda et al., 2023). Octadecanoic acid, also known as stearic acid when it forms an ester like octadecanoic acid, ethyl ester, exhibits several significant biological activities as antioxidant and anti-inflammatory (Ganesh & Mohankumar, 2017). Hexadecanoic acid as a potent antibacterial compound against multidrug-resistant (MDR) strains such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The mechanism involves disrupting bacterial cell membranes and impairing energy generation, leading to cell lysis (Nabi et al., 2022). In addition to its antimicrobial effects, hexadecanoic acid also exhibits antioxidant activities. Studies have reported its ability to scavenge free radicals, which contributes to its potential use in pharmaceuticals aimed at reducing oxidative stress (Ganesan et al., 2024).

Antimicrobial Activity

The results of antimicrobial activity testing of Ethanol Extract of Jamblang Stem Bark (EEJSB) against *C. acnes*, *S. aureus*, and *C. albicans* with various concentrations using disc diffusion method can be seen in Figure 2. When the discs containing EEJSB have been treated and incubated for 24 hours, the jamblang bark extract will diffuse to the surface of the media to inhibit the growth of the microorganisms. This can be seen from the formation of a inhibition zone around the disk on the media that has been overgrown with microorganisms. The formation of a inhibition zone proves that the extract has inhibited microbial growth. The clear zone is a clear area around the disk of microbial growth media that is not overgrown by microbes (Mengko et al., 2022).

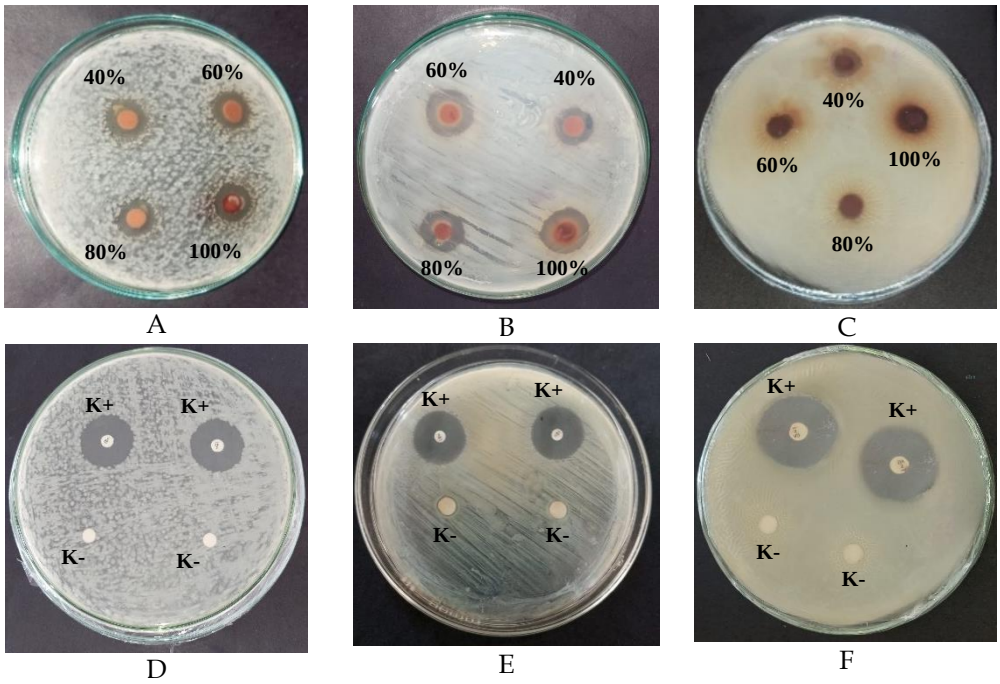

Figure 2. Antimicrobial activity of ethanol extract of jamblang stem bark (EEJSB) (A). EEJSB against *C. acnes*, (B). EEJSB against *S. aureus*, (C). EEJSB against *C. albicans*, (D). Clindamycin (K+) and 96% ethanol (K-) against *C. acnes*, (E). Clindamycin (K+) and 96% ethanol (K-) against *S. aureus*, (F). Chloramphenicole (K+) and 96% ethanol (K-) against *C. albicans*

The results of the study in Table 2, show that testing variations in the concentration of EEJSB can inhibit the growth of the microorganisms tested. Antimicrobial testing of EEJSB with a concentration of 100% produced the largest average clear zone diameter against *S. aureus* bacteria, followed by *C. acnes* and *C. albicans* with inhibition zone diameters of 8.04 mm, 7.45 mm, and 4.60 mm, respectively.

Table 2. Antimicrobial activity of ethanol extract of jamblang stem bark

EEJSB	Diameter inhibition zone *Cutibacterium acnes* (mm)	Diameter inhibition zone *Staphylococcus aureus* (mm)	Diameter inhibition zone *Candida albicans* (mm)
40%	5.02 ± 0.77	3.47 ± 1.35	2.27 ± 1.63
60%	6.67 ± 1.24	4.86 ± 1.39	2.95 ± 1.52
80%	7.05 ± 1.45	5.71 ± 1.39	3.19 ± 1.94
100%	7.45 ± 1.17	8.04 ± 1.20	4.60 ± 1.43
Control +	20.70 ± 0.45	22.29 ± 0.19	18.62 ± 0.51
Control -	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

The zone of inhibition of EEJSB against *C. acnes* bacteria with concentrations of 40%, 60%, 80%, and 100% is categorized as moderate. The zone of inhibition of EEJSB against *S. aureus* bacteria with concentrations of 40% and 60% was classified as weak, while EEJSB concentrations of 80%, and 100% were classified as moderate. Meanwhile, the inhibition zone formed against *C. albicans* fungi at all EEJSB concentrations was categorized as weak. The classification of the inhibition zone is based on the statement of Morales et al. (2003)who divided the microbial growth inhibition response based on the diameter of the inhibition zone into four groups, namely <5 mm categorized as weak, 5 mm to 10 mm categorized as moderate, 11-20 mm categorized as strong, and >20 mm categorized as very strong.

Antibacterial test of jamblang leaf extract against *C. acnes* bacteria conducted by Setiawan et al. (2024)gave results of inhibition zone diameter ranging from the range of 7 mm to 9 mm, which is also classified in the medium category. Meanwhile, the testing of jamblang bark extract against *S. aureus* bacteria conducted by Utang et al. (2020) produced an inhibition zone diameter of 5 mm to 7 mm which is also categorized as weak-medium. Antifungal testing of jamblang bark extract conducted by Utang against *Aspergillus niger* fungi also had weak potential with inhibition zone length ranging from 2.5 mm to 4.0 mm. Even research conducted by Rasnovi & Nursanty (2014) showed that n-hexane

extract of jamblang stem could not inhibit the growth of fungi *Candida* sp.

Table 2 also shows that the higher the concentration of EEJSB given to the isolates of *C. acnes*, *S. aureus*, and *C. albicans*, the greater the clear zone formed. The results of this study are in accordance with research conducted by Sudarmi et al. (2017), which showed that increasing the concentration of jamblang leaf extract (*S. cumini*) caused an increase in the diameter of the inhibition zone. Similarly, as revealed by Sudarwati & Sumarni (2016), that usually the concentration of extract used is directly proportional to the inhibition zone produced, this happens because the higher the concentration used, the more antimicrobial substances contained in it.

The ability of EEJSB to inhibit the growth of *C. acnes, S. aureus*, and *C. albicans* is due to activities that can inhibit the formation of microbial cell walls, inhibit the permeability of microbial cell walls, inhibit enzyme action, and inhibit the synthesis of nucleic acids and proteins (Angelina, 2022). The inhibition occurs due to the content of secondary metabolites in jamblang bark, as stated by Mukhlis, (2011) and Jagetia et al. (2017) that jamblang bark contains flavonoids, tannins, saponins, alkaloids, friedelin, friedelan-3-α-ol, betulinic acid, β-sitosterol, kaempferol, β-sitosterol-D-glucoside, gallic acid, elagic acid, gallic tannin, and mirisetin which act as antimicrobials. Secondary metabolites contained in plants serve to protect themselves from external disturbances of the surrounding environment.

Flavonoid compounds are known as antibacterial against pathogenic microorganisms. The mechanism of flavonoids as antibacterial is by inhibiting the movement of bacteria, increasing the action of antibiotics, weakening pathogenic bacteria (Xie et al., 2015). Ngajow et al. (2013) explained the mechanism of action of tannins as antibacterial is by targeting the polypeptide wall so that cell wall formation becomes incomplete, causing cells to lysis and triggering bacterial cell death. There are also saponin compounds, saponins can cause disruption of cell membrane work so that bacterial growth can be inhibited. This compound can cause a decrease in surface tension on the cell wall, so that the cell membrane loses its stability and causes disruption of ion transport (Vagestini et al., 2023). The mechanism of action of alkaloids as antibacterials is by disrupting the components that make up the peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes cell death (Rijayanti et al., 2020).

The length of the inhibition zone diameter from each test gives different results. This difference is influenced by several factors such as the type of microbes inhibited, the concentration of the extract, the content of antimicrobial compounds, the diffusion power of the extract, and the structure of the microbial cell wall (Egra et al., 2019). C. *acnes* and *S. aureus* bacteria are Gram-positive bacteria whose cell walls are simpler than the cell walls of *C. albicans* fungi, so secondary metabolite compounds are easier to enter bacterial cells than fungi. Therefore, the results of this test prove that EEJSB has more potential as an antibacterial than antifungal.

The growth of *Candida albicans* can be inhibited properly if the amount of secondary metabolite compounds in the extract is more and more specific than the compounds needed to inhibit other microbes because the defense system of *Candida albicans* is quite strong. The defense of this fungus can be seen from the structure of the cell wall which consists of 5 layers, then has a plasma membrane layer whose outer part consists of lipids and the presence of ergosterol which is a double phospholipid membrane that can withstand lysis due to osmotic pressure. The nature of *Candida albicans* at 37 ℃ can form Clamydiospora which has a very thick and strong spore wall that is difficult to penetrate (Rizky et al., 2023).

The negative control test results on each test microbe showed a value of 0.00 mm, indicating that the formation of an inhibition zone around the disc containing EEJSB did not come from the 96% ethanol solvent used during maceration. Ethanol 96% is an organic solvent that does not interfere with the bioactivity of secondary metabolites against pathogenic bacteria (Khalil, 2013). Although this EEJSB test was shown to have antibacterial and antifungal activity, the activity was still lower than the positive control which had strong to very strong antibacterial and antifungal activity.

Conclusion

Based on the GC-MS analysis, the dominant compound identified in the Ethanol Extract of Jamblang Stem Bark (EEJSB) is 7-Tetradecenal (Z)-, which belongs to the aldehyde class and is known to have potential biological activity as antioxidant and anti-inflammatory. Antimicrobial testing showed that the higher the concentration of EEJSB used, the larger the diameter of the inhibition zone formed, indicating an increase in antimicrobial effectiveness. The best results were obtained against *Staphylococcus aureus* bacteria with a clear zone of 8.04 mm, followed by *Cutibacterium acnes* at 7.45 mm, and *Candida albicans* at 4.60 mm. These findings suggest that EEJSB has potential as an antibacterial agent, particularly against bacteria that cause skin diseases, with varying effectiveness depending on the type of target microorganism.

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Author Contributions
FM, YSI, and LF were involved in concepting and planning the research, FM performed the data acquisition/collection, FM calculated the experimental data and performed the analysis, FM drafted the manuscript and designed the figures, FM aided in interpreting the results. All authors took parts in giving critical revision of the manuscript.

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Conflicts of Interest
The authors declare no conflict of interest.

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