Berita Biologi
LIPI
Jurnal ilmu-ilmu hayati

Berita Biologi 23(1): 61-71 (2024)
https://ejournal.brin.go.id/berita_biologi ISSN 2077 7019
DOI: 10.55981/beritabiologi.2024.3618

P-ISSN 0126-1754
E-ISSN 2337-8751

ARTIKEL

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF ETHYL ACETATE EXTRACT OF ACTINOMYCETES ISOLATED FROM TERMITE NESTS

[*Aktivitas Antioksidan dan Antibakteri Ekstrak Etil Asetat Actinomycetes yang Diisolasi dari Sarang Rayap*]

Ahmad Fathoni[1*], Andi Saptaji Kamal[1], Lukman Hafid[1], Lina Marlina[1], Oscar Efendy[1], Ade Lia Putri[2], Praptiwi[1], Andria Agusta[1,3]

[1]Research Center for Pharmaceutical Ingredient and Traditional Medicine, National Research and Innovation Agency (BRIN), Cibinong, Bogor 16911, Indonesia.
[2]Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Cibinong, Bogor 16911, Indonesia.
[3]Faculty Medicine, Malahayati University, Jl. Pramuka No.27, Kemiling Permai, Bandar Lampung, Lampung 35152, Indonesia.

ABSTRACT

Actinomycetes are an important source for the discovery of bioactive secondary metabolite compounds. Over 10,000 bioactive metabolite compounds have been isolated from terrestrial actinomycetes with various biological activities, such as antibiotic, antiviral, anti-inflammatory, antitumor, anticancer, and antioxidant. One source of origin for actinomycetes is soil, including termite nests. Utilization of natural sources such as actinomycetes from termite nests as antioxidants and antibacterials can be an alternative source of production of new secondary metabolite compounds. This research aims to evaluate the antioxidant and antibacterial activities of actinomycete extracts from termite nests. Antioxidant activity was observed using the DPPH free radical scavenging method, while antibacterial activity was measured by observing the growth inhibition of *Staphylococcus aureus*. Antioxidant and antibacterial activity were performed by using the TLC dot-blot, TLC bioautography, and microdilution methods to determine the inhibitory concentration of 50% (IC_{50}), antioxidant activity index (AAI), and minimum inhibitory concentration (MIC). From the 33 extracts tested, 16 extracts showed antioxidant activity with IC_{50} values range of 76.64-126.22 µg/mL or AAI value > 0.05 (moderate), and 8 extracts had moderate antibacterial activity against *S. aureus* (MIC values range of 256-512 µg/mL). Future research for scaling up of actinomycete culture, isolating active compounds, determining the chemical structure of active compounds, and further testing as antioxidants and antibacterials still need to be carried out.

Keywords: antioxidant, antibacterial, actinomycetes, termite nests

Diterima: 12 Februari 2024; **Diperbaiki:** 15 Februari 2024; **Disetujui:** 21 Maret 2024
***Penulis untuk Korespondensi:** e-mail – ahma060@brin.go.id

ABSTRAK

Actinomycetes merupakan sumber penting bagi penemuan senyawa metabolit sekunder bioaktif. Lebih dari 10.000 senyawa metabolit bioaktif telah diisolasi dari actinomycetes terestrial dengan berbagai aktivitas biologis, seperti antibiotik, antivirus, antiinflamasi, antitumor, antikanker, dan antioksidan. Salah satu sumber asal actinomycetes adalah tanah, termasuk sarang rayap. Pemanfaatan sumber alam seperti aktinomisetes dari sarang rayap sebagai antioksidan dan antibakteri dapat menjadi alternatif sumber produksi senyawa metabolit sekunder baru. Penelitian ini bertujuan untuk mengevaluasi aktivitas antioksidan dan antibakteri ekstrak actinomycete dari sarang rayap. Aktivitas antioksidan menggunakan metode penangkap radikal bebas DPPH, dan aktivitas antibakteri diamati menggunakan bakteri Staphylococcus aureus. Aktivitas antioksidan dan antibakteri dilakukan dengan menggunakan metode KLT dot-blot, KLT bioautografi, dan mikrodilusi untuk menentukan konsentrasi hambat 50% (IC50), indeks aktivitas antioksidan (AAI), dan konsentrasi hambat minimum (MIC). Dari 33 ekstrak yang diuji, 16 ekstrak menunjukkan aktivitas antioksidan dengan rentang nilai IC_{50} yaitu 76,64-126,22 µg/mL atau nilai AAI > 0,05 (sedang), dan 8 ekstrak memiliki aktivitas antibakteri sedang terhadap S. aureus (nilai KHM berkisar 256-512 µg/mL). Penelitian selanjutnya untuk peningkatan kultur aktinomiset, isolasi senyawa aktif, penentuan struktur kimia senyawa aktif, dan pengujian lebih lanjut sebagai antioksidan dan antibakteri masih perlu dilakukan.

***Kata kunci**: antioksidan, antibakteri, actinomycetes, sarang rayap*

INTRODUCTION

Actinomycetes are an important source for the discovery of bioactive secondary metabolite compounds. More than 10,000 bioactive metabolite compounds have been isolated from terrestrial Actinomycetes with various biological activities, such as antibiotic, antiviral, anti-inflammatory, antitumor, anticancer, and antioxidant (Av-Gay *et al*., 2007). Over the last few decades, various new bioactive compounds from terrestrial Actinomycetes have been isolated and clinically tested (Chin *et al*., 2006). For example, daptomycin, miglustat, and amrubycin are several derivatives or analogs of compounds isolated from Actinomycetes and have been used as drugs. However, currently, the progress in the discovery of new bioactive compounds from Actinomycetes tends to decrease, while the rate of reisolation of known bioactive compounds is increasing. Considering this, it is necessary to look for new sources of Actinomycetes to obtain secondary metabolite compounds with specific bioactivity.

Actinomycetes are prokaryotic organisms including gram-positive, free-living, saprophytic bacteria, widely distributed in soil and water, and colonize plant tissues or endophytes, and also produce various active compounds from secondary metabolism. The morphological characteristics of these prokaryotes resemble fungi because they have hyphae or filaments but are not insulated, however, these microbes are included in the bacteria group because they are prokaryotes and contain peptidoglycan in their cell walls (Fatmawati *et al*., 2014).

Termite nests contain material that comes from dirt, where dirt can easily become germs and cause disease when in a warm, moist, and closed environment. According to Chouvenc (2013), termite feces produce natural antibiotics. Dirt grains contain actinomycetes, such as Streptomyces, that contain certain compounds. The compounds of Streptomyces can inhibit the growth of fungi that are harmful to termite colonies. Previous studies examined the microbial biota within the termite gut, and actinobacteria were identified as one of the dominant bacteria in this symbiotic lifestyle (Khucharoenphaisan *et al*., 2012; Matsui *et al*., 2012; Sujada *et al*., 2014). These symbiotic actinomycetes provide assisting functions for termites, such as nutrient cycling and exchange and also protect termites from invading pathogens. Some of these termite-associated actinomycetes may also exhibit lignin-cellulolytic activity and antagonistic activity against diverse pathogens (Sujada *et al*., 2014)). Actinomycetes are known as antimicrobial producers and also produce antioxidant compounds (Rammali *et al*., 2022). Utilization of termite nest Actinomycetes as antioxidants and antibacterials can increase the production source of new secondary metabolite compounds.

The use of Actinomycetes in termite nests as a source of antibacterial and antioxidant compounds has the prospect of developing them as phytopharmaceuticals or ingredients needed in the food industry or others. In the health sector, natural antibacterial compounds are urgently needed

due to the increasing number of pathogenic bacteria that are resistant to existing antibiotics. Natural antioxidant compounds are needed to prevent degenerative diseases, such as coronary heart disease, cancer, and others. In the food industry to prevent and inhibit food products from contamination by pathogenic bacteria and oxidation of proteins or fats, to delay deterioration in quality and preserve food products. This research is aimed to evaluate the antioxidant and antibacterial activity of actinomycete extracts from termite nests. Antioxidant and antibacterial activity were carried out using the TLC method (dot-blot and bioautography), and microdilution assay to determine the 50% inhibitory concentration (IC50), antioxidant activity index (AAI), and minimum inhibitory concentration (MIC).

MATERIALS AND METHODS

Material

Termite nests were collected in Kebun Raya Indonesia, Bogor, West Java, Indonesia.

Isolation of Actinomycetes

The termite nest samples were air-dried for five days, then pulverized, and filtered through a 2 mm mesh. Actinomycetes were isolated using the Sodium Dodecyl Sulphate-Yeast Extract (SDS-YE) (Praptiwi *et al*., 2019): a gram of soil particles was mixed with 9 ml of sterile aquadest, vortexed for 10 minutes, and 0.5 ml of this mixture was added to SDS-YE in a test tube. After another round of vortexing for 3 minutes, the suspension was incubated in a water bath shaker at 40°C for 20 minutes. Following incubation, the suspension was diluted and cultured on Humic Acid-Vitamin Agar (HVA) supplemented with a mixture of antibiotics (nalidixic acid; $10mg.L^{-1}$ and chlortetracycline; $10mg.L^{-1}$) in Petri dishes, incubating at 30°C for 1-2 weeks. Actinomycetes colonies from the HVA plates were further purified on Yeast Extract media and incubated for 1-2 weeks. Purified actinomycetes isolates were preserved in 10% glycerol at -80°C for future use.

Actinomycetes Cultivation

Actinomycetes (1 x 1 cm^2 in size) were cultured in liquid media such as YIM 310 liquid media (Praptiwi *et al*., 2023) containing of glucose 5.0 g; peptone 3.0 g; soluble starch 10.0 g; yeast extract 3.0 g; $CaCO_3$ 2.0 g; NH_4NO_3 3.0 g in 1000 mL water, and placed on a shaker incubator set at 130 rpm, maintaining a temperature range of 22-24°C for 14 days.

Extraction of Bioactive Metabolites of Actinomycetes

Following a two-week incubation period, the culture media and actinomycetes biomass underwent extraction using ethyl acetate three times (Praptiwi *et al*., 2023). The ethyl acetate extract was concentrated using a rotary evaporator and then preserved at -4°C for future utilization.

Chemical Compounds Analysis by Using Thin Layer Chromatography

Chemical compound analysis (Fathoni *et al*., 2021) of actinomycetes extracts was conducted using Thin Layer Chromatography (TLC) on a silica plate (Merck GF254). A volume of ten microliters of the extract (at a concentration of 10 mg/ml) was applied to the silica plate and allowed to dry. Subsequently, the plate was subjected to development in a solution of dichloromethane and methanol (in a 10:1 ratio). Observation of the plate was carried out under UV light at wavelengths of 254 nm and 366 nm. Following this, the plate was sprayed with vanillin sulfate as a staining reagent.

Screening of Antibacterial by Using TLC Dot-Blot

Fifty-one actinomycetes extracts were assessed for antibacterial against *S. aureus* InaCC-B4 (Fathoni *et al*., 2021) using thin layer chromatography (TLC) bioautography. Ten microliters of extracts at a concentration of 10 mg/ml were applied to TLC plates. Once the application was complete, the plates were dried and immersed in a bacterial suspension, followed by incubation at 37°C for 18 hours. Post-incubation, the plates were sprayed with a solution of iodo nitro tetrazolium

chloride (INT) (4 mg/ml; Sigma). Active antibacterial extracts were identified by the presence of clear zones around the areas where the extracts were applied.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC determination for the active antibacterial of the extracts involved microdilution in 96-well plates with three repetitions (Fathoni *et al*., 2022). Initially, each well in the first row (A1-A12) contained 100 µL of Mueller Hinton Broth with two times concentration (MHB2x), 10 µL of extract (at 10.24 mg/mL in DMSO), and 90 µL of sterile aquadest, thoroughly mixed. Subsequent rows (B to D) contained 100 µL of MHB 1x. Serial dilutions were performed by transferring 100 µL from the first row to the second and homogenizing the contents. This process generated a concentration range of 128-1024 µg/mL. The bacterial suspension (100 µL) was then added to each well, and the microplate was incubated at 37°C for 24 hours under aseptic conditions using a laminar flow hood. Following incubation, 10 µL of iodo nitro tetrazolium (INT) chloride solution (4 mg/mL) was added to each well. The lowest concentration where the color did not change to red indicated the Minimum Inhibitory Concentration (MIC).

Screening of Antioxidant by Using TLC-Dot Blot

The evaluation of actinomycetes extracts for antioxidant potential as 2,2-Diphenyl-1-picrylhydrazyl (DPPH)-free radical scavengers involved a bioautography technique on a silica plate (Merck GF254) (Fathoni *et al*., 2023). Ten microliters of extracts (at a concentration of 10 mg/ml) were applied onto the TLC plate. Once the application was completed, the TLC plate was treated with a DPPH solution in methanol (0.2 mg/mL; Sigma) by spraying. The assessment of antioxidant activity was conducted 10 minutes post-spraying with the DPPH solution. Extracts demonstrating antioxidant properties were recognized by developing a yellowish area surrounding the applied extract spots.

Determination of IC_{50} Values

To assess the IC_{50} values of active antioxidant extracts (Fathoni *et al*., 2023), a 96-well microplate was used with an initial 100 µL of methanol in each well. The process began by adding 5 µL of the extract (at 10.24 mg/mL) and 95 µL to the first row, mixing thoroughly. Sequentially, a dilution sequence was carried out by transferring 100 µL from the first row to the next, repeating this pattern for subsequent rows. Following the dilution, 100 µL of a DPPH solution (61.5 µg/mL) was introduced into each well. The microplate was then kept at room temperature in darkness for 90 minutes. Measurement of absorbance at 517 nm using a microplate reader (Variate Flash, Thermo Scientific) allowed determination of the IC_{50} value based on the absorbance levels.

IC value (%) = $(A_{DPPH100}-A_{Extract})*100/A_{Extract}$

Where: IC value= percentage of inhibitory concentration; $A_{DPPH\ 100}$ = absorbance of blank samples with DPPH solution in methanol; $A_{Extract}$ = absorbance of extracts with DPPH solution in methanol.

The IC_{50} value (The 50 % inhibitory concentration of DPPH) was calculated based on a linear regression equation between extract concentration and IC percentage. The antioxidant activity index (AAI) is as follows:

AAI = [Final concentration of DPPH]/IC_{50}

RESULTS

Chemical Compounds Analysis: Thin Layer Chromatography

Chemical compound analysis of actinomycetes from Termite nests was conducted using Thin Layer Chromatography (TLC) on silica plates (Merck, GF254) (Figure 2). The visualization of chemical components can be done either under UV light or detected by staining spray reagents. The compounds visualized under UV light 366 nm appeared as bright bands with a fluorescent effect on the dark blue background. While the substances were monitored under UV light 254 nm appeared as dark bands on a bright background.

The chemical components of actinomycete extracts according to the color spots appeared on the TLC plate that was monitored under UV light and after being sprayed with staining reagents. Identification based on color on appearance under UV in 254 and 366 nm as well as with sprayed staining reagents of cerium (IV) sulfate and vanillin-H_2SO_4 from literature (Fathoni et al., 2021).The chemical components appeared blue fluorescence under UV light 366 nm to identify coumarins (such as No. 17; *Rf*: 0.4; 0.5; 0.6 and No 32; *Rf*: 0.6) and dark spots under UV 254 nm to identify for coumarins (such as No. 32; *Rf*: 0.6) and terpenoids (such as No. 32; *Rf*: 0.4). After sprayed with vanillin-sulfuric acid staining reagent, the TLC spots showed the presence of terpenoids (dark green, orange, purple, maroon, or brown color spots such as sample No. (No. 32; *Rf*: 0.4, brown color (Figure 2).

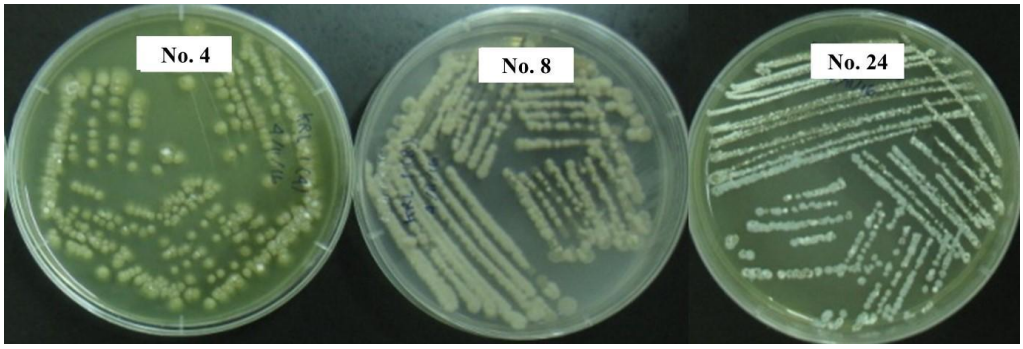

Figure 1. Several isolates of actinomycetes from termite nest. Notes: Isolate No. 4 (KRI-1-04); 8 (KRI-1-08) and 24 (KRI-1-24). (*Gambar 1. Beberapa isolat actinomycetes dari sarang rayap. Catatan : Isolat* No. 4 (KRI-1-04); 8 (KRI-1-08) *dan* 24 (KRI-1-24)).

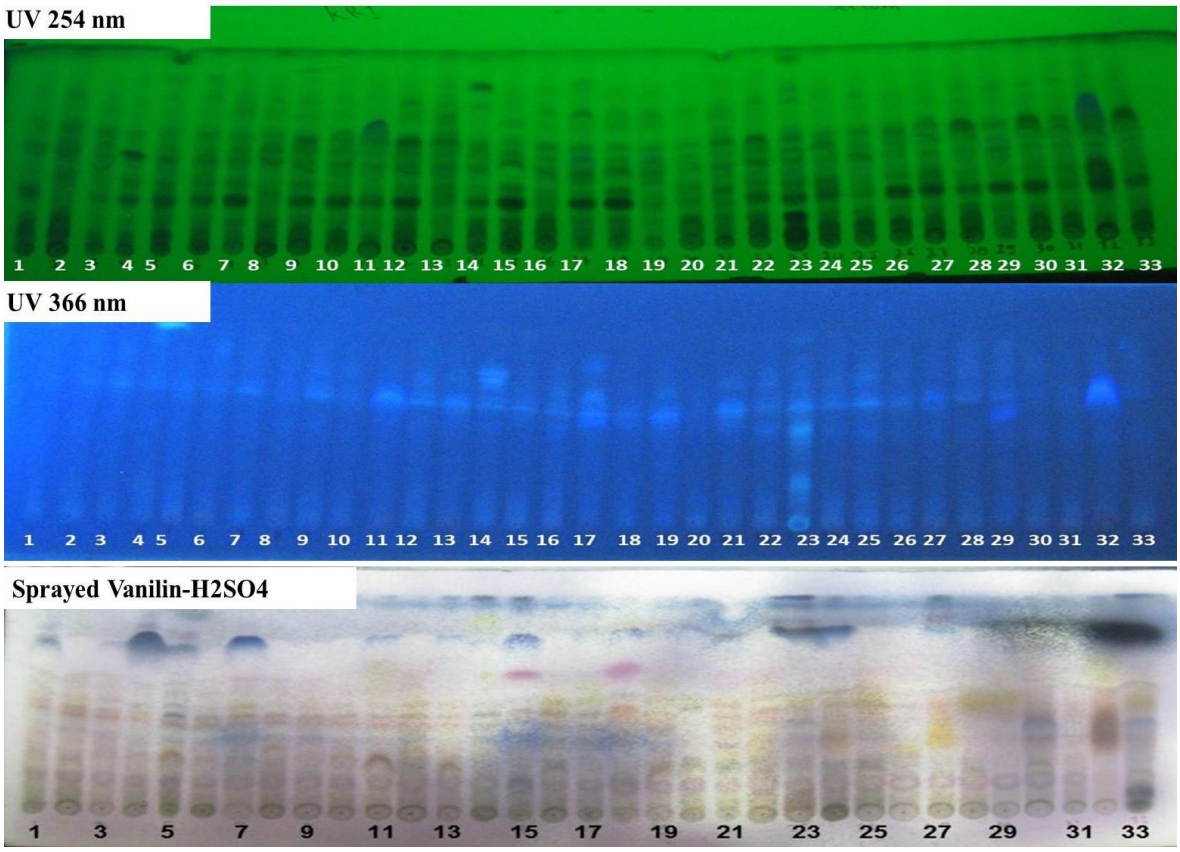

Figure 2. Chromatograms of The EtOAc Extracts of Actinomycetes. (*Gambar 2. Kromatogram Ekstrak EtOAc Actinomycetes*)

Screening of Antibacterial and Antioxidant Activity: TLC-Dot Blot

Several isolates of actinomycetes are shown in Figure 1. A total of 33 actinomycetes extracts were evaluated for antibacterial effects against *S. aureus* Ina CC-B4 using TLC-dot blot (Table 1). The results of the study demonstrate the potential antioxidant and antibacterial activities of various extracts. The antibacterial activity against *S. aureus* was evaluated using TLC Dot-Blot assays, and 10 extracts displayed antibacterial properties. While the DPPH radical scavenging assay revealed that 25 extracts exhibited as antioxidants.

The combination of antioxidant and antibacterial activities in these extracts could be attributed to the presence of secondary metabolites such as coumarins and terpenoids (such as No. 17 and 32) or other bioactive compounds (Figure 3 and 4). These compounds are known for their diverse biological activities, including antioxidant and antimicrobial properties (Tsivileva *et al.*, 2022; Masyita *et al.*, 2022).

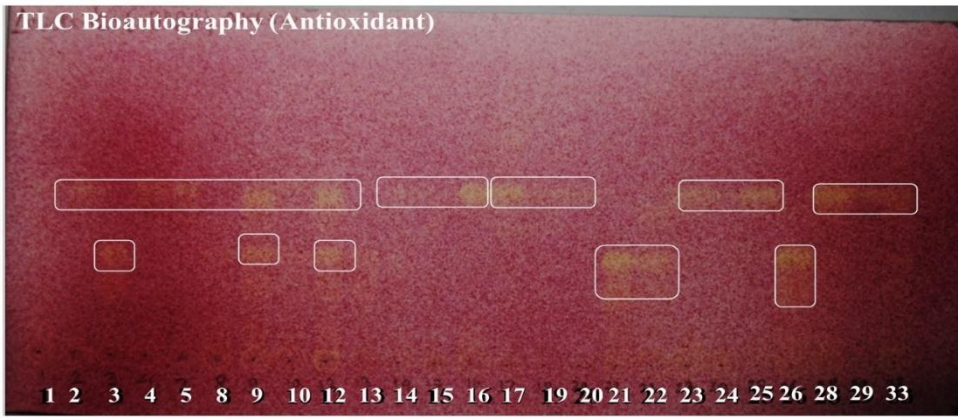

Figure 3. TLC Bioautography as antioxidant of active extract of Actinomycetes. (*Gambar 3. KLT Bioautografi sebagai antioksidan ekstrak aktif Actinomycetes*).

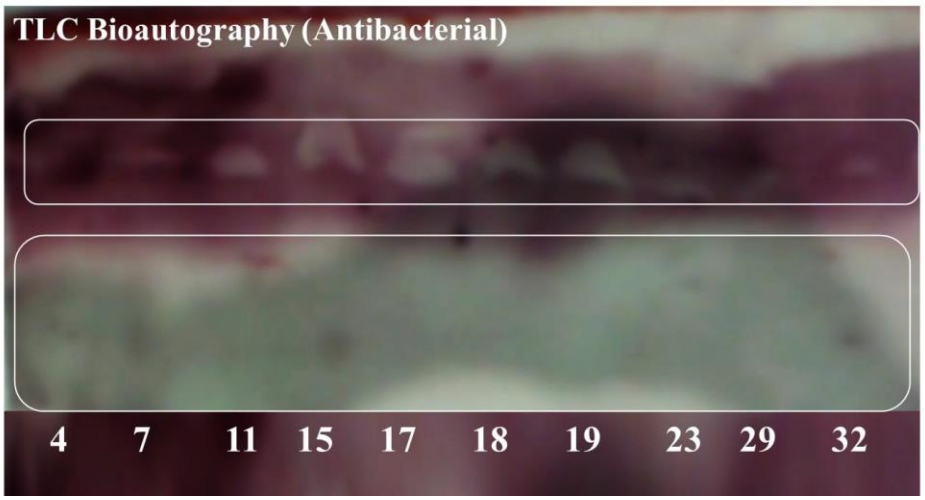

Figure 4. TLC Bioautography as antibacterial of active extract. (*Gambar 4. KLT Bioautografi sebagai antibakteri ekstrak aktif*)

Determination of Minimum Inhibitory Concentration (MIC) of Extracts

The MIC values of the actinomycetes extracts against *S. aureus* ranged from 256 to 10240 µg/mL. Criteria of MIC values for extracts: inactive <1000 µg/ml <weak activity< 625µg/ml < moderate < 100 µg/ml < significant (Kuete, 2010). In this study, 8 extracts showed moderate antibacterial activity against *S. aureus*, as their MIC values were 128-1024 µg/ml.

Antibacterial activity observed might be related to the chemical compounds present in the extracts such as samples No. 17 and 32 containing coumarins and terpenoids based on TLC assay (Figure 2) which contributes as an antibacterial with moderate activity (Table 1). Future studies could delve into the identification and characterization of the active compounds responsible for antibacterial activity.

Screening of Antioxidant Activity: TLC-Bioautography and Determination of IC_{50} Value

Screening of antioxidant activity of 33 actinomycetes extracts was carried out by TLC-Dot-Blot assay. In this method, the active antioxidant extract was indicated by, a yellowish spot formation around the extract on the purple background. White-yellowish spot formation because the extract can reduce DPPH free radical to yellow-colored diphenyl-picrylhydrazine because of its ability to donate hydrogen. The yellow color intensity can be used as an indication of antioxidant capacity. From TLC-Dot Blot revealed that 25 extracts have activity as antioxidants (Table 1), and further investigation for active extracts with microdilution methods for determining IC_{50} and AAI values.

Scherer and Godoy (2009)] classified the antioxidant activity of the extract based on the AAI value as follows: poor activity<0.05<moderate<1.00<strong<2.00<very strong. Further investigation showed that 16 extracts were classified as moderate antioxidants (AAI values > 0.05) such as extract No.17 with an IC_{50} value of 126.22 µg/mL or AAI value of 0.24 (Table 1). This suggests a moderate ability of these extracts to neutralize free radicals.

Table 1. Extract Production and Their Bioactivities. (*Tabel 1. Produksi Ekstrak dan Bioaktivitasnya*).

No	Isolate Code	Extract Weight (mg)	Antioxidant as DPPH radical free scavenging				Antibacterial against *S.aureus*		
			TLC Dot Blot	IC_{50} (µg/mL)	AAI	Category	TLC Dot Blot	MIC (µg/mL)	Category
1	KRI-1-01	200.1	+	93.95	0.33	Moderate	-	NT	NA
2	KRI-1-02	217.5	+	116.64	0.26	Moderate	-	NT	NA
3	KRI-1-03	165.7	+	101.23	0.30	Moderate	-	NT	NA
4	KRI-1-04	280.7	+	121.47	0.25	Moderate	+	512	Moderate
5	KRI-1-05	404.1	-	NT	NT	NA	-	NT	NA
6	KRI-1-06	170.4	-	NT	NT	NA	-	NT	NA
7	KRI-1-07	217.4	+	114.32	0.27	Moderate	+	512	Moderate
8	KRI-1-08	261.8	+	120.97	0.25	Moderate	-	NT	NA
9	KRI-1-09	189.4	+	**77.31**	0.40	Moderate	-	NT	NA
10	KRI-1-10	175.6	+	>128	<0.24	Weak-Moderate	-	NT	NA
11	KRI-1-11	156.4	-	>128	<0.24	Weak-Moderate	+	512	Moderate
12	KRI-1-12	183.1	+	104.19	0.30	Moderate	-	NT	NA
13	KRI-1-13	169.7	+	>128	<0.24	Weak-Moderate	-	NT	NA
14	KRI-1-14	198.2	+	>128	<0.24	Weak-Moderate	-	NT	NA
15	KRI-1-15	374.2	+	>128	<0.24	Weak-Moderate	+	512	Moderate
16	KRI-1-16	212.3	+	>128	<0.24	Weak-Moderate	-	NT	NA
17	KRI-1-17	140.9	+	126.22	0.24	Moderate	+	**256**	Moderate
18	KRI-1-18	159,20	-	NT	NA	NA	+	1024	Weak
19	KRI-1-19	100.7	+	121.47	0.25	Moderate	+	1024	Weak
20	KRI-1-20	135.2	+	>128	<0.24	Weak-Moderate	-	NT	NA
21	KRI-1-21	208.0	+	>128	<0.24	Weak-Moderate	-	NT	NA
22	KRI-1-22	167.2	+	122.38	0.25	Moderate	-	NT	NA
23	KRI-1-23	195.8	+	101.59	0.32	Moderate	+	512	Moderate
24	KRI-1-24	244.1	+	>128	<0.24	Weak-Moderate	-	NT	NA
25	KRI-1-25	170.6	+	122.92	0.25	Moderate	-	NT	NA
26	KRI-1-26	140.6	+	94.67	0.40	Moderate	-	NT	NA
27	KRI-1-27	124.2	-	NT	NT	NA	-	NT	NA
28	KRI-1-28	214.5	+	96.35	0.32	Moderate	-	NT	NA
29	KRI-1-29	141.4	+	>128	<0.24	Weak-Moderate	+	512	Moderate
30	KRI-1-30	196.4	-	NT	NT	NA	-	NT	NA
31	KRI-1-31	149.9	-	NT	NT	NT	-	NT	NA
32	KRI-1-32	131.7	-	>128	<0.24	Weak-Moderate	+	512	Moderate
33	KRI-1-33	196.0	+	**76.64**	0.40	Moderate	-	NT	NA

Remark: (-): Not Active, (+): Active, NT: Not Tested, NA: Not Active

DISCUSSION

Based on Chemical analysis using Thin Layer Chromatography (TLC) on silica plates (Merck, GF_{254}) showed actinomycetes extracts have diverse compounds such as coumarins and terpenoids. This method is recognized for its quick, cost-effective, and convenient separation of compounds on a flat surface, allowing for simultaneous analysis of multiple samples. TLC relies on compound polarity, causing different compounds to bind to the absorbent with varying strengths, resulting in distinct migration patterns on the silica plate. Multiple spots representing various compounds within one actinomycete extract were observed in Figure 2, each exhibiting different migration patterns. Stained reagents such as vanillin sulfate were used to visualize these spots, potentially indicating the class of chemical compounds present. Compounds appeared as blue fluorescence under UV light at 366 nm, indicating the presence of coumarins, while under UV light at 254 nm, dark spots indicated the presence of coumarins and terpenoids. After spraying with vanillin-sulfuric acid staining reagent, the TLC spots showed the presence of terpenoids, visible as dark green, orange, purple, maroon, or brown color spots.

Several actinomycetes extracts also have antibacterial effects against *S. aureus* Ina CC-B4 using TLC-dot blot and microdilution assay as well as an antioxidant as DPPH radical free scavenging with moderate activity (Table 1). This technique, known as TLC-dot blot, not only offers several benefits including its simplicity, fast, requirement of small sample quantities, and straightforward interpretation of results but also allows simultaneous analysis of multiple samples and specifically targets active chemical compounds. The appearance of a clear zone around the extracts on a purple background is indicative of inhibition of bacterial growth. On the other hand, an antioxidant test assay is commonly used to assess the ability of compounds to neutralize free radicals, and the active extracts likely contain compounds capable of donating electrons to the DPPH radicals, thereby reducing their activity (Table 1). It's noteworthy that the use of TLC Dot-Blot assays allowed for a visual representation of the antibacterial and antioxidant activity, making it easier to identify active extracts.

From MIC value of several actinomycetes extracts have antibacterial with moderate category. These suggest that actinomycetes extracts have promising antibacterial properties against *S. aureus*. Additionally, further investigating the specific mechanisms of action and the spectrum of activity against other bacterial strains would contribute to a more comprehensive understanding of their therapeutic potential. From IC_{50} and AAI values of antioxidant activity of the extracts, possible compounds that act as antioxidants and antibacterials such as coumarin in endophytic fungus (such as extract no. 17 at Rf 0.6 in Figure 3 and 4). Other research states that coumarins have properties as antiviral, antimicrobial, antioxidant, anti-inflammatory, antiadipogenic, cytotoxic, apoptotic, antitumor, antitubercular, and cytotoxicity agents (Tsivileva et al., 2022).

The study reveals the antibacterial and antioxidant activities of actinomycetes extracts, with a notable subset showing moderate antibacterial effects against *S. aureus*. The connection between these activities and the chemical composition of the extracts provides a foundation for future research and potential applications in medicine or biotechnology.

CONCLUSION

The study assessing the potential of actinomycetes isolated from termite nests can be summarized that eight extracts of actinomycetes had moderate antibacterial activity against *S. aureus* (MIC values range of 128-1024 μg/mL). Sixteen extracts had moderate antioxidant activity with IC_{50} (76.64-126.22 μg/mL) or AAI values > 0.05. The results showed that actinomycetes isolated from termite nests might be used as promising sources of antibacterial and antioxidant. Further study needs to be done to isolate the antibacterial or antioxidant active compounds in the active extract.

ACKNOWLEDGEMENT

This study was supported by the Japan-ASEAN Science, Technology, and Innovation Platform (JASTIP) and the Research Center for Pharmaceutical Ingredient and Traditional Medicine, National Research and Innovation Agency (BRIN). The author thanks Kiagus M. Fathi for assisting in laboratory work.

AUTHORSHIP CONTRIBUTION

All authors are the main contributors to this paper. The authorship contributions such as AF: conceptualization of the study, analysis of data, and drafting of the manuscript. ASK, LH, and LM: collection and analysis of data. OE: sampling, data collection, and analysis. ALP: isolation and identification of microbial specimens, as well as manuscript preparation. P: data collection and manuscript writing. AA: conceptualization of the study, supervision, and manuscript preparation.

REFERENCES

Av-Gay Y, Rawat M. 2007. Mycothiol-dependent proteins in *Actinomycetes*. *FEMS Microbiology Reviews*. 31(3), pp. 278-292. Doi: 10.1111/j.1574 6976.2006.00062.x.

Chin YW, Balunas MJ, Chai HB, Kinghorn AD. 2006. Drug discovery from natural sources. *The AAPS Journal*. 8, pp. 239-253

Chouvenc T, Caroline A, Efstathion, Monica LE. 2013. Extended disease resistance emerging from the faecal nest of a subterranean termite. *Proceedings of The Royal Society B.* Department of Entomology and Nematology University of Florida Institute of Food and Agricultural Sciences.

Fathoni A, Hudiyono S, Budianto E, Cahyana AH, Agusta A. 2021. Metabolite Detection and Antibacterial Activity of Fungal Endophytic Extracts Isolated from Brotowali (Tinospora crispa) Plants using TLC-Bioautography Assay. *IOP Conf. Ser.: Mater. Sci. Eng.* 1011: 012041

Fathoni A, M Ilyas, Praptiwi, D Wulansari, A Agusta. 2022. Antibacterial and Antioxidant Activities of Fungal Endophytes Isolated from Medicinal Plants in Simeulue Island, Aceh. *HAYATI Journal of Biosciences*, 29(6), pp. 720-732

Fathoni A, S Hudiyono, AH Cahyana, M Ilyas, I Purnaningsih, A Agusta. 2023. Anticandidal and antioxidant potencies of endophytic fungi associated with *Tinospora crispa*. *Biodiversitas,* 24(5), pp. 2547-2555

Fatmawati U, Santosa S, Rinanto Y, Probosari RM. 2014. Aktivitas antibakteri *Actinomycetes* yang diisolasi dari rizosfer tanaman *solanaceae. Jurnal Farmasi Indonesia.* 11(1), pp. 54-68. ISSN: 1693-8615. EISSN: 2302-4291

Jiang Y, Q. Li, X. Chen and C. Jiang, 2016. Isolation and Cultivation Methods of Actinobacteria, In: Actinobacteria-Basics and Biotechnological Applications, edited by Dharumadurai Dhanasekaran and Jiang Y (IntechOpenLimited, London), pp. 39-57

Khucharoenphaisan K, Sripairoj N, Sinma K. 2012. Isolation and identification of actinomycetes from termite's gut against human pathogen. *Asian J Anim Vet Adv.*7, pp. 68–73.

Kuete V. 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. *Planta Med.* 76(14), pp. 1479-1491. doi: 10.1055/s-0030-1250027.

Masyita, A., Sari, R.M., Astuti, A.D., Yasir, B., Rumata, N.R., Emran, T.B., Nainu, F., Simal-Gandara, J. 2022.Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. *Food Chemistry*, 10(13), pp. 100217.

Matsui T, Tanaka J, Namihira T, Shinzato N. 2012. Antibiotics production by an actinomycete isolated from the termite gut. *J Basic Microbiol*, 52, pp. 1–5.

Praptiwi, M Raunsai, D Wulansari, A Fathoni, A Agusta. 2019. Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi. *Journal of Applied Pharmaceutical Science,* 8(8), pp. 69-74.

Praptiwi, A Fathoni, AL Putri, D Wulansari, A Agusta. 2023. Biological potency of actinomycetes extracts from rhizosphere soil of *Dacrycarpus imbricatus* from Toba Samosir, North Sumatra. *Journal of Applied Pharmaceutical Science,* 13(06), pp. 146-153.

Rammali, S., Hilali, L., Dari, K. *et al.* 2022. Antimicrobial and antioxidant activities of *Streptomyces* species from soils of three different cold sites in the Fez-Meknes region Morocco. *Sci Rep,* 12, pp. 17233. https://doi.org/10.1038/s41598-022-21644-z

Sujada N, Sungthong R, Lumyong S. 2014. Termite nests as an abundant source of cultivable actinobacteria for biotechnological purposes. *Microbes Environ*. 29(2), pp. 211-219. doi: 10.1264/jsme2.me13183.

Scherer R, and Godoy HT. 2009. Antioxidant activity index (AAI) by the 2,2-diphenyl-1-picrylhydrazyl method. Food Chemistry, 112(3), pp. 654-658.

Tsivileva OM, Koftin OV, Evseeva NV. 2022. Coumarins as Fungal Metabolites with Potential Medicinal Properties. Antibiotics (Basel)11(9), pp. 1156. doi: 10.3390/antibiotics11091156.