Jurnal Veteriner pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional Peringkat 2, Dirjen Riset dan Pengembangan,
Kemendiktisaintek RI SK No 10/C/C3/DT.05.00/2025, 21 Mar.2025

Juni 2025 Vol. 26 No.2: 163-174
DOI: 10.19087/jveteriner.2025.26.2.163
online pada http://ojs.unud.ac.id/index.php/jvet

Screening of Cytotoxic Activity of Soluble Pigment Biomolecules from Actinomycetes Isolated from Various Locations in South Sulawesi

(PENAPISAN AKTIVITAS SITOTOKSIK BIOMOLEKUL PIGMEN TERLARUT ASAL AKTINOMISETES YANG DIISOLASI DARI BERBAGAI LOKASI DI SULAWESI SELATAN)

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ABSTRACT

The diversity of colors in metabolites, particularly soluble pigments secreted by actinomycetes, has been minimally reported as cytotoxic metabolites. This study was aimed to screen the cytotoxic activity of secondary metabolites, especially soluble pigments produced by tropical actinomycetes. Actinomycetes metabolites were produced through batch fermentation using 100 mL of Starch Nitrate broth media in 500 mL Erlenmeyer flasks. The resulting ethyl acetate extracts were tested for cytotoxicity against the Michigan Cancer Foundation-7 (MCF-7) cell line. The characterization of isolates was based on colony morphology and spore chain analysis. The results of this study showed that the cytotoxic activity of secondary metabolites against cancer cells (specifically MCF-7) was as follows: 30.016 µg/mL for isolate Py/kM (yellow pigment), 27.53 µg/mL for isolate Ac-e/b (pink pigment), 35.559 µg/mL for isolate SM-r/B (brown pigment), 33.636 µg/mL for isolate Lc-r/F (purple pigment), and 30.986 µg/mL for isolate Dis 014 (orange pigment).

Keywords: Actinomycetes; biomolecules; soluble pigment; cytotoxic

ABSTRAK

Keragaman warna dalam metabolit, khususnya pigmen larut yang disekresikan oleh aktinomisetes, telah dilaporkan sebagai metabolit sitotoksik. Penelitian ini bertujuan untuk menyaring aktivitas sitotoksik metabolit sekunder, khususnya pigmen larut yang diproduksi oleh aktinomisetes tropis. Metabolit aktinomisetes diproduksi melalui fermentasi batch menggunakan 100 mL media Starch Nitrate broth dalam labu Erlenmeyer 500 mL. Ekstrak etil asetat yang dihasilkan diuji untuk sitotoksisitas terhadap lini sel Michigan Cancer Foundation-7 (MCF-7). Karakterisasi isolat didasarkan pada morfologi koloni dan analisis rantai spora. Hasil penelitian menunjukkan bahwa aktivitas sitotoksik metabolit sekunder terhadap sel kanker (khususnya MCF-7) adalah sebagai berikut: 30,016 µg/mL untuk isolat Py/kM (pigmen kuning), 27,53 µg/mL untuk isolat Ac-e/b (pigmen merah muda), 35,559 µg/mL untuk isolat SM-r/B (pigmen coklat), 33,636 µg/mL untuk isolat Lc-r/F (pigmen ungu), dan 30,986 µg/mL untuk isolat Dis 014 (pigmen jingga).

Kata-kata kunci: aktinomiset; biomolekul; pigmen terlarut; sitotoksik

INTRODUCTION

It is estimated that there were 12 million new cancer cases and 7.6 million cancer-related deaths in 2008, with the incidence expected to rise to 26.4 million new cases annually worldwide by 2030, resulting in 17 million deaths (Davies-Bolorunduro *et al*., 2019). In 2023, 1,958,310 new cancer cases and 609,820 cancer-related deaths were projected to occur in the United States. The incidence of prostate cancer increased by 3% annually from 2014 to 2019 after a two-decade decline, representing an additional 99,000 new cases. Lung cancer in women decreased at half the rate of men (1.1% vs. 2.6% annually) from 2015 to 2019, while cancer of the corpus uteri and breast continued to rise, as well as liver cancer and melanoma, both of which remained stable (Siegel *et al*., 2023).

This situation highlights the importance of various efforts to develop anticancer drugs as treatment options. One approach targets natural products from microorganisms such as bacteria, fungi, plants, and animals. Approximately 23,000 secondary metabolites from microorganisms are known, with actinomycetes exclusively producing about 42%, while fungi produce a nearly equal amount (42%), and 16% of the remaining metabolites are produced by eubacteria (Guo *et al*., 2015; Selim *et al*., 2021). Microbial secondary metabolites, including growth hormones, pigments, antibiotics, and antitumor agents, do not directly influence the growth and development of the microorganisms but have proven to be crucial in medical treatments and human health (Lapenda *et al*, 2020).

Actinomycetes are bacteria found in various environments. These microorganisms are responsible for the production of most of the molecules available in the market and produce important antitumor agents used in cancer treatment (Guimarães *et al*., 2020). Several metabolites such as rubromycin, which is cytostatic to various cancer cells (MCF-7, HMO2, KatoIII, and HEPG2) (Lin *et al.*, 2022), the compound 1(10-aminodecyl) pyridinium (against HeLa, MCF-7, and U87MG) (Dasari *et al*., 2012), and ethyl acetate metabolites from the genera *Strep-tomyces* and *Nocardiopsis* (against MDA-MB-231) (Abdelfattah *et al.*, 2016) are examples of such bioactive compounds.

Breast cancer cells, particularly those classified as MCF-7 cells, are one of the primary targets in cancer research due to their high prevalence and the urgent need for more effective therapies. The MCF-7 cell line originates from human breast cancer and is commonly used as an *in vitro* model to evaluate the anticancer activity of various compounds. Cytotoxicity testing using these cells can provide valuable preliminary information about the therapeutic potential of the compounds or extracts being tested (Abraham and Chauhan, 2018).

In recent decades, much has been reported regarding the anticancer activity of actinomycetes isolated from various environments. It has been reported that more than 10,000 bioactive secondary metabolites are produced by actinomycetes, with 7,600 compounds generated by the *Streptomyces* genus, accounting for 45% of all bioactive microbial metabolites discovered (Rani *et al*., 2021). One important biomolecule produced by actinomycetes is pigments, which are known to have several biological activities such as cytostatic and cytotoxic effects.

This study focuses on the cytotoxicity testing of ethyl acetate extracts from secondary metabolites produced by actinomycetes using MCF-7 cells. The primary objective of this research was to evaluate the effectiveness of these extracts in inhibiting the growth of breast cancer cells and to identify potential bioactive compounds that may be further developed as anticancer agents.

RESEARCH METHODS

Isolation and Selection of Actinomycetes Strains

Actinomycetes strains were isolated from various locations in South Sulawesi and are part of the collection of the Microbiology Laboratory (Microbial Diversity Research Division) at Department of Biology, Faculty of Mathematics and Natural Sciences, UNM. These strains were cultivated on Starch Nitrate Agar (SNA) medium. Colonies producing soluble pigments were selected for secondary metabolite production.

Production of soluble pigment metabolites

All selected actinomycetes strains were pre-cultured in 250 mL Erlenmeyer flasks containing 50 mL of liquid SNB medium and incubated at 30°C for five days. The production of soluble pigment metabolites was conducted by transferring the pre-culture (starter) into 1000 mL Erlenmeyer flasks containing 250 mL of SNB medium (20 g soluble starch, 2 g KNO_3, 2 g NaCl, 0.05 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeSO_4 \cdot 7H_2O$, 2 g K_2HPO_4, and 1 L distilled water). Fermentation was carried out at 30°C for 10 days under agitation at 150 rpm.

After fermentation, the microbial growth medium was filtered to separate the biomass from the culture liquid. The culture liquid was extracted with ethyl acetate (1:1 v/v) using a separatory funnel and allowed to stand for 15 minutes. The resulting extract was dissolved again in ethyl acetate, dried, and stored in a desiccator for subsequent analysis. Cytotoxic activity was determined using the Methyl Tiazolydiphenyl Tetrabromide (MTT) assay to calculate the minimum concentration of metabolites required to inhibit 50% (IC_{50}) of the cell line.

Cytotoxicity Assay Against MCF-7 Cells

Preparation of Solutions. The ethyl acetate extract obtained was dissolved in dimethyl sulfoxide (DMSO) and serially diluted in the culture medium to prepare a range of concentrations.

Cell Viability. Cell viability was assessed using the MTT assay. Confluent MCF-7 cells were harvested and distributed into 96-well microplates at a density of 5×10^3 cells per well. The cells were incubated for 48 hours in a carbon dioxide (CO_2) incubator to allow adaptation before treatment. Following treatment, cells were incubated for an additional 24 hours in the CO_2 incubator. After incubation, the test solution was removed, and 100 µL of MTT reagent was added to each well. A stopper reagent was added after three hours of incubation with MTT. The plate was incubated overnight at room temperature in the dark. At the end of the incubation period, the plate was shaken on a horizontal shaker for 10 minutes and read using an Enzyme-Linked Immunosorbent Assay (ELISA) reader at 595 nm.

Cytotoxicity Analysis of Soluble Pigment Metabolites

Cytotoxicity was assessed using a modified method. The MCF-7 cells (5×10^3 cells per well) were cultured in 96-well plates containing media with varying extract concentrations [500 – 250 – 125 – 62.5 – 31.25 µg/mL]. The cells were incubated at 37°C with 5% CO_2, 95% air, and 100% relative humidity. After the incubation period, the medium was removed, and 100 µL of medium containing 1 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was added to each well. The cells were cultured for four hours, after which the medium was removed, and 100 µL of DMSO was added to dissolve the crystals. Cytotoxicity against cancer cells was determined by measuring the absorbance of the converted dye at 570 nm using an ELISA reader.

The cytotoxicity of each sample was expressed as the IC_{50} value. The IC_{50} value represents the concentration of the test sample that inhibits 50% of cell growth, averaged from three experimental replicates and calculated using the formula: Cell Viability (%) = [(Absorbance of treated cells – Absorbance media control) X (Absorbance of cell control – Absorbance of media control)$^{-1}$] × 100

The percentage of cell viability data was then used to calculate the IC_{50} value through linear regression analysis of the logarithmic concentration of the test sample versus the percentage of cell viability.

RESULTS AND DISCUSSION

Bacterial Strains

This study was involved the isolation of actinomycetes strains from various natural sources, including plants and animals. Specifically, the strains were

isolated from common lantana **(***Lantana camara)***,** citronella grass **(***Cymbopogon nardus)***,** fragrant poikilospermum *(Poikilospermum suaveolens)***,** bulb onion *(Allium cepa)***,** and marine sponges (*Porifera* sp.). The sample sources varied and included the rhizosphere, plant roots, and marine sponges. In this study, the isolated actinomycetes were exhibited the ability to produce soluble pigments in various colors, such as purple, yellow, brown, and pink (Table 1).

Characteristics of Actinomycete Strains

The morphological analysis of the strains was found to reveal diverse characteristics. For instance, strain Py/kM was observed to exhibit distinctive morphological features, including the formation of orange, carpet-like substrate mycelia, which is a typical trait of the genus *Streptomyces*. The aerial mycelium of this strain was white, aligning with the general description of *Streptomyces*, which is characterized by often having aerial mycelia contrasting with their substrate mycelia.

Strain Ac-e/b was found to display pink substrate mycelia with gray aerial mycelia. The pink coloration of the substrate mycelia is indicative of the potential production of specific pigments, which are believed to exhibit unique biological activities. *Streptomyces* is well-known for being able to produce diverse pigments, and the pink substrate is indicative of a distinctive potential of this strain. Strain SM-r/B was observed to form colonies with brown substrate mycelia and white aerial mycelia. Strain Lc-r/F was found to exhibit purple substrate mycelia with cream-colored aerial mycelia. Strain Dis 014 was characterized by forming colonies with orange substrate mycelia and white aerial mycelia, which are similar to the characteristics of strain Py/kM (Figure 1).

Cell Viability of MCF-7 Treated with Actinomycete Strains and Doxorubicin

The viability curves of MCF-7 cells treated with selected actinomycete strains and doxorubicin are presented in Figure 2. Treatments were conducted at concentrations of 500, 250, 125, 62.5 and 31.25 µg/mL. All tested strains Py/kM, Ac-e/b, SM-r/B, Lc-r/F, and Dis 014 exhibited a dose-dependent cytotoxic effect on MCF-7 cells, as indicated by decreasing cell viability with increasing concentrations. As expected, doxorubicin, the positive control, demonstrated the most potent cytotoxic effect with a substantially lower IC_{50} value (0.5 µg/mL), reducing cell viability sharply even at the lowest tested concentrations. In contrast, the untreated MCF-7 control maintained 100% viability across all conditions, confirming the absence of non-specific cytotoxicity.

Cytotoxicity Testing of Metabolites

The cytotoxicity test results on the MCF-7 cell line showed that the ethyl acetate extracts of metabolites from various actinomycetes strains exhibited varying activities in inhibiting breast cancer cell growth. This cytotoxic activity was measured through the IC_{50} value, which represents the concentration of the extract required to inhibit 50% of cell growth.

The metabolite extract from strain Py/kM was found to have an IC_{50} value of 30.016 ± 2.71 µg/mL, indicating that the extract from this strain has significant cytotoxic potential**.** The metabolite extract from strain Ac-e/b demonstrated the lowest IC_{50} value among the tested strains, at 27.53 ± 1.595 µg/mL, signifying that the extract from this strain possesses the highest cytotoxic potential against MCF-7 cells. A lower IC_{50} value indicates greater cytotoxic potential, making the Ac-e/b extract the most prominent in inhibiting the growth of MCF-7 breast cancer cells compared to the other strains.

The metabolite extract from strain SM-r/B was observed to have the highest IC_{50} value among the tested strains, at 35.559 ± 1.81 µg/mL, indicating lower cytotoxic activity compared to the others. Strain Lc-r/F showed an IC_{50} value of 33.636 ± 2.773 µg/mL, which also indicated moderate cytotoxic potential.

Table 1. Characteristics of actinomycete strains producing soluble pigments isolated from various types of organisms

Strains	Plant/animal	Sample source	Soluble pigment color
Py/kM	*Poikilospermum suaveolens*	Plant root	Yellow/orange
Ac-e/b	*Allium cepa*	Plant root	Pink
SM-r/B	*Cymbopogon nardus*	Rhizosphere	Brown
Lc-r/F	*Lantana camara*	Rhizosphere	Purple
Dis 014	Spons	Spons	Oranges

Figure 1. Morphological characteristics of the pigment-producing strain on Starch Nitrate Agar medium, incubated for seven days at 30°C.

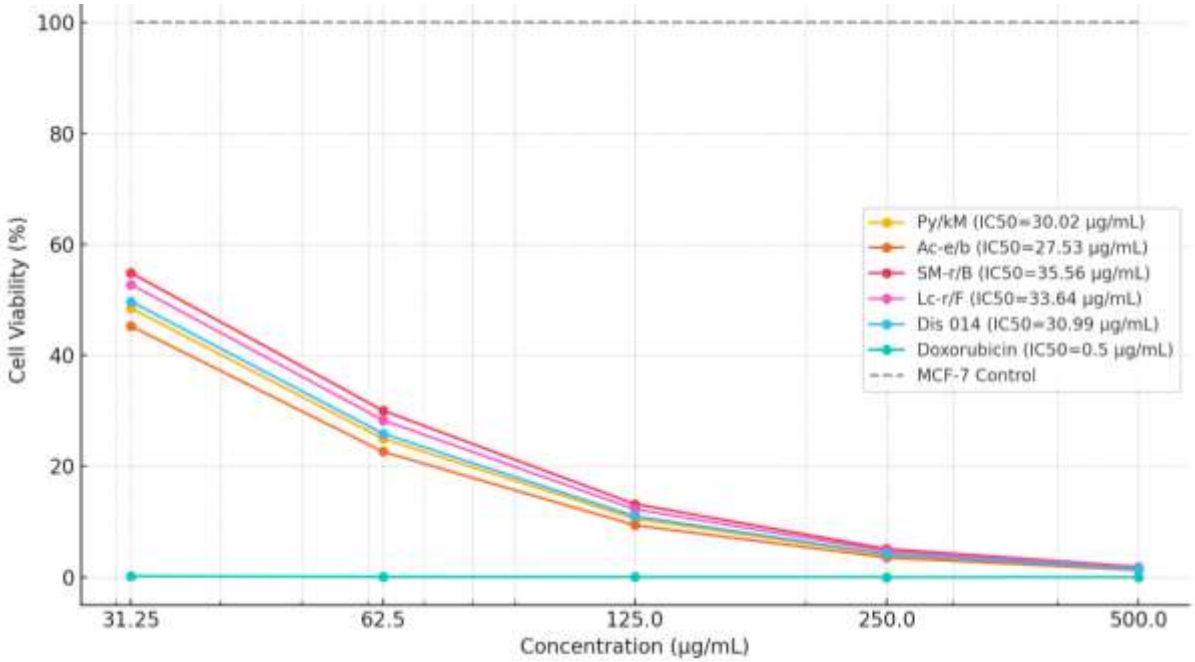

Figure 2. MCF-7 cell viability curves treated with Actinomycete strains at selected concentrations

Table 2. Cytotoxic activity of ethyl acetate extracts of soluble pigment secondary metabolites from actinomycete strains

Strains	IC_{50} (µg/mL)
Py/kM	30.02 ± 2.71

Ac-e/b	27.53 ± 1.59
SM-r/B	35.56 ± 1.81
Lc-r/F	33.64 ± 2.77
Dis 014	30.99 ± 1.35

Additionally, strain Dis 014 was found to have an IC_{50} value of 30.986 ± 1.348 μg/mL, which is close to the IC_{50} value of strain Py/kM, suggesting similar cytotoxic potential (Table 2).

In this study, observations were conducted on the density profile and morphology of MCF-7 cells treated with action-mycetes extracts. The MCF-7 cells exhibited a significant reduction in density compared to the control. Moreover, the morphology of the cells treated with actinomycetes extracts underwent changes, becoming more irregular in shape, with indications of apoptosis such as nuclear fragmentation. The MTT assay was employed to measure cell viability following treatment with actinomycetes extracts. Medium controls and cell controls were used as comparisons. The results of the MTT assay were demonstrated that the viability of MCF-7 cells treated with actinomycetes extracts decreased signifycantly compared to both the medium control and the cell control (Figure 3).

GC/MS Aalysis of Metabolites from Actinomycete Strains

The GC/MS analysis of actinomycetes strain metabolites revealed that the active compound responsible for inhibiting cell line growth **is** cis-5,8,11,14,17-eicosapentaenoic acid (Figure 4, 5, 6). Additionally, another compound, cis-13-eicosenoic acid, was also identified, along with the chemical structures of each compound.

The research on natural compounds has become a major focus in the development of new drugs, particularly in the field of oncology (Newman and Cragg, 2020). One potential source of bioactive compounds is microorganisms, including actinomycetes. Actinomycetes are Gram-positive bacteria well-known for their ability to produce a wide range of secondary metabolites with significant biological activities (Barka *et al*., 2016; Ser *et al*., 2016). These secondary metabolites, including antibiotics, anticancer agents and immunomodulatory compounds, have demonstrated great potential in various medical applications (Atanasov *et al*., 2021).

The isolation of actinomycetes from various natural sources highlights the diversity of habitats these microorganisms occupy. Actinomycetes are predominantly found in various ecological habitats, including marine ecosystems such as water bodies, coral reefs, seawater, and mangrove forests (Liu *et al***.**, 2015). Actinomycetes are among the microorganisms commonly encountered in diverse soil types, often constituting a significant portion of the soil microflora. They are widely distributed in nature, particularly in soil and constitute a significant portion of the telluric microflora (Sethi *et al*., 2021). Plants such as *Lantana camara*, *Cymbopogon nardus*, *Poikilospermum suaveolens*, and *Allium cepa*, as well as marine sponges, provide unique environments for the growth and development of actinomycetes. Marine actinomycetes associate with a variety of aquatic organisms, including invertebrates such as sponges, corals and echinoderms (Xu *et al.*, 2021)

The rhizosphere and plant roots provide a nutrient-rich microenvironment that supports the growth of microorganisms that produce valuable secondary metabolites (Mendes *et al*., 2013; Dinesh *et al*., 2012). This microenvironment is crucial for the survival and metabolic activity of microorganisms, including actinomycetes, which are known to produce a variety of bioactive compounds. The isolation of actinomycetes from marine sponges is also significant, as sponges are known to harbour symbiotic relationships with various microorganisms, including actinomycetes, which contribute to the production of unique bioactive compounds (Blunt *et al*., 2013). The symbiosis between actinomycetes and marine sponges enhances the production of secondary metabolites, offering potential for the discovery of novel bioactive compounds with various therapeutic applications.

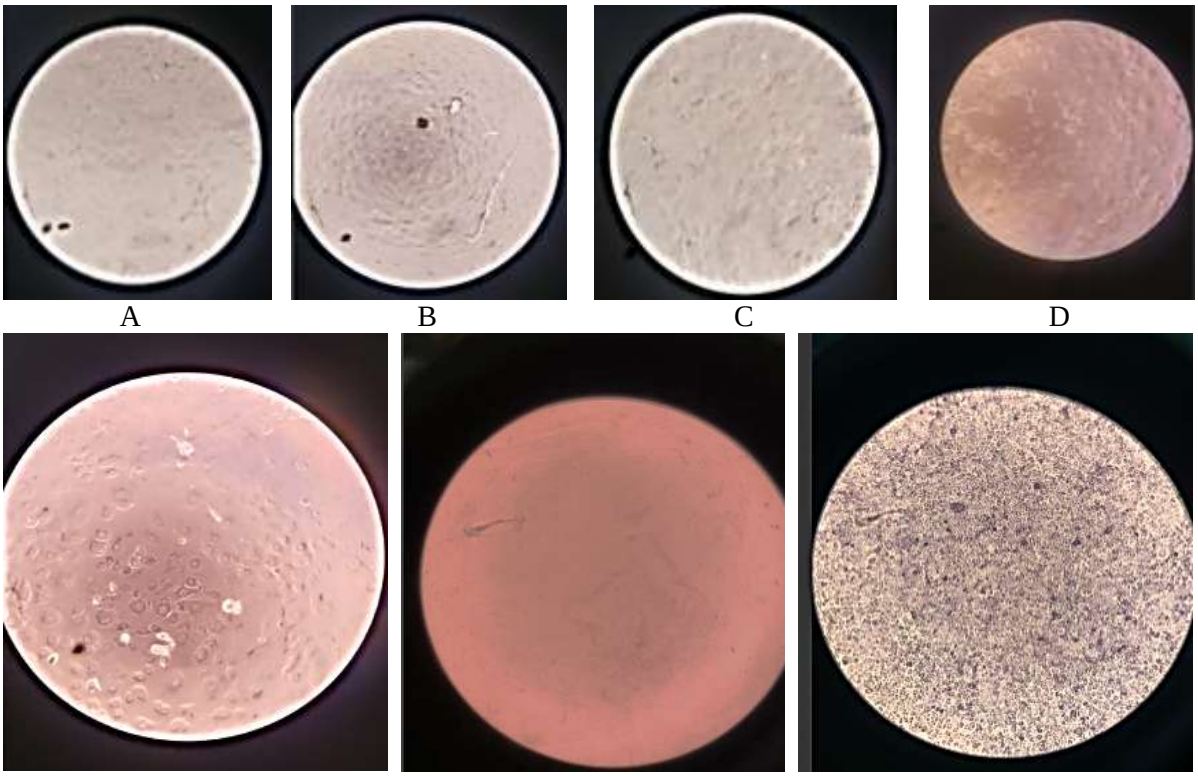

Figure 3. Density and morphology of MCF-7 cells, (A) treated with Py/kM strain extract, (B) treated with Ac-e/b strain extract, (C) treated with SM-r/B strain extract, (D) treated with Lc-r/F strain extract, (E) treated with Dis 014 strain extract, (F) medium control after MTT assay treatment, (G) cell control after MTT assay treatment, observed under a microscope at 100X magnification.

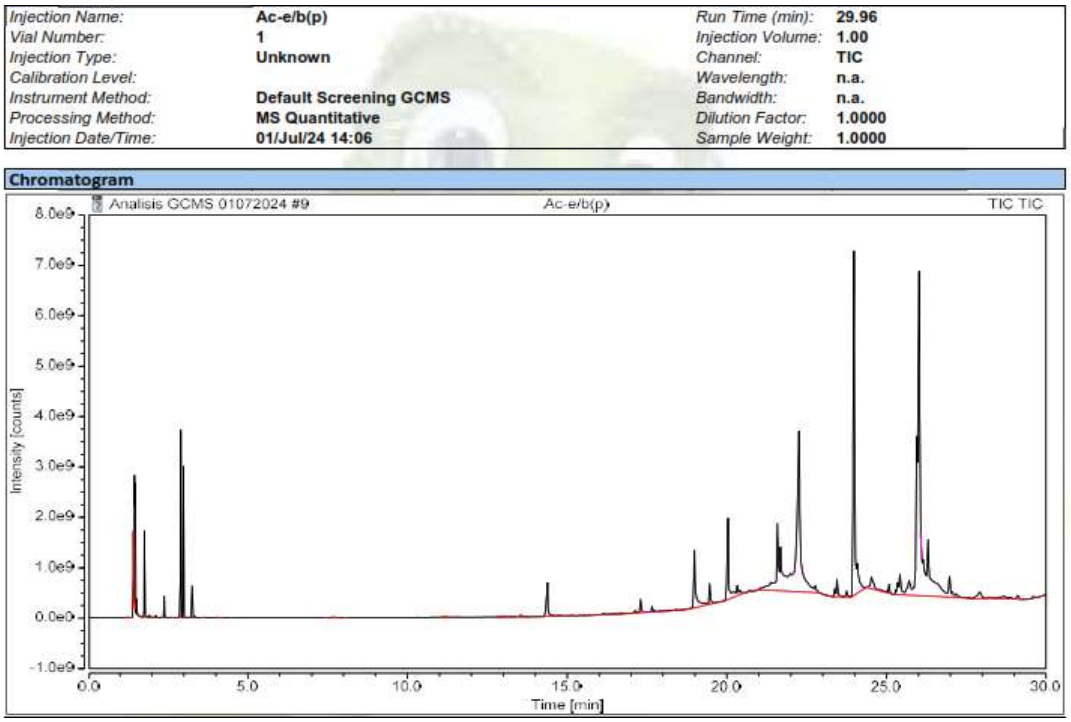

Figure 4. Chromatogram of metabolites from strain Ac-e/b based on GC-MS analysis

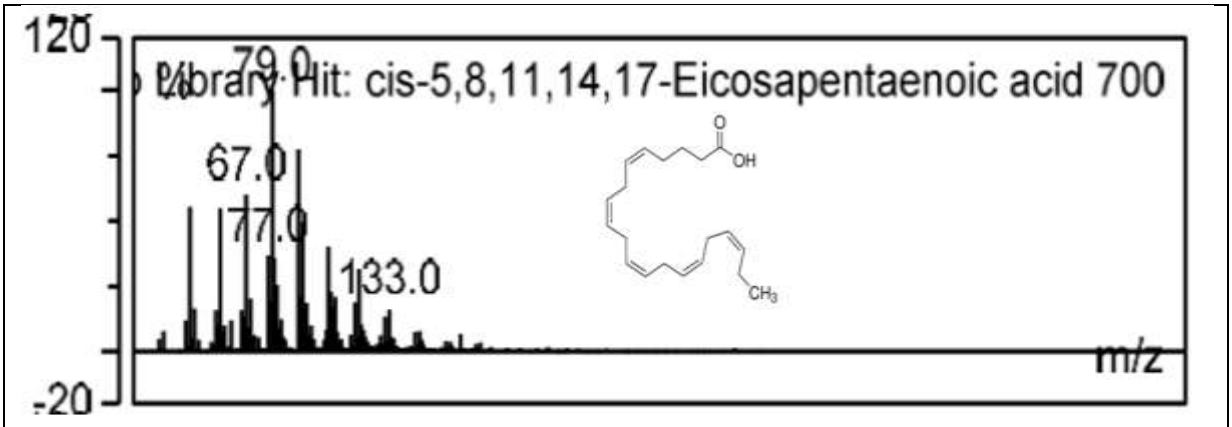

Figure 5. Chromatogram and chemical structure of cis-5,8,11,14,17-eicosapentaenoic acid, a compound inhibiting cell line growth

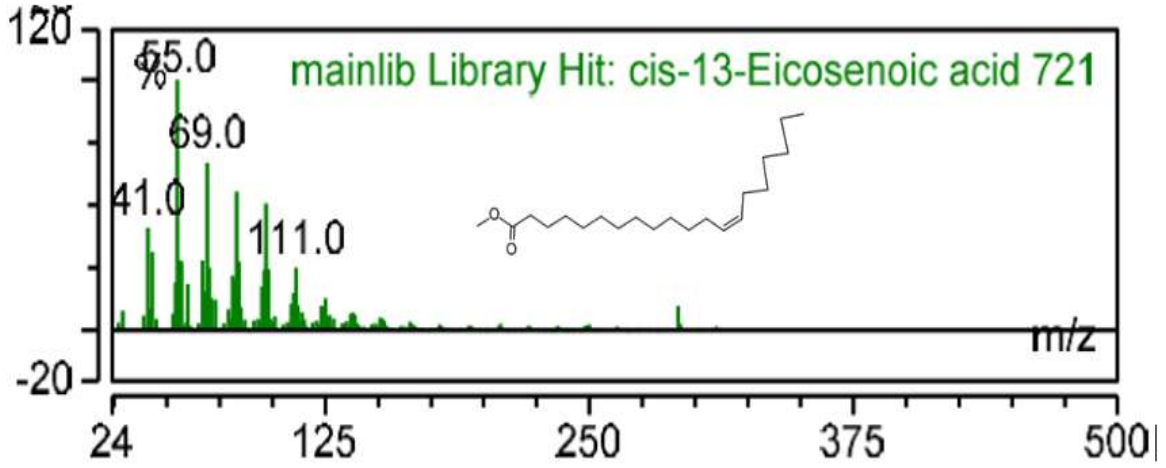

Figure 6. Chromatogram and chemical structure of cis-13-eicosenoic acid, a compound inhibiting cell line growth

The production of soluble pigments by actinomycetes strains isolated from various sources demonstrates their diverse metabolic capabilities. Pigments such as purple, yellow, brown and pink indicate that these actinomycetes have the ability to produce a wide range of chemical compounds, which may have industrial and medical applications. These pigments not only serve as indicators of the presence of secondary metabolites but may also possess valuable biological properties, such as antibacterial, antifungal, or anticancer activities (Soliev *et al*., 2011; Pelay-Ruiz *et al.,* 2019). The ability of actinomycetes to produce such pigments reflects their metabolic diversity, which could be further explored for the development of novel therapeutic agents. Overall, the diversity of sources and pigment production capa-bilities suggests the great potential of the isolated actinomycetes as a source of secondary metabolites with various applications, including the development of anticancer agents, as demonstrated in cytotoxicity tests using the MCF-7 cell line.

The cytotoxicity test against the MCF-7 cell line demonstrated that the ethyl acetate extracts of metabolites from various actinomycetes strains exhibit varying levels of activity in inhibiting the growth of breast

cancer cells. This cytotoxic activity was measured by the IC_{50} value, which represents the concentration of the extract required to inhibit cell growth by 50%. The IC_{50} value is a crucial parameter often used in pharmacological studies to assess the anticancer potential of a compound or extract (Berdy, 2005).

The cytotoxic analysis of the metabolite extract from the Py/kM strain revealed significant cytotoxic potential. In the context of oncology, an IC_{50} value within this range indicates that the Py/kM strain produces secondary metabolites that effectively inhibit the proliferation of breast cancer cells. Similarly, the metabolites from the Ac-e/b strain showed the lowest IC_{50} value among the tested strains. This value suggests that the extract from this strain possesses the highest cytotoxic potential against the MCF-7 cells. A lower IC_{50} value signifies greater cytotoxic potency, meaning that the Ac-e/b extract has the most prominent activity in inhibiting the growth of MCF-7 breast cancer cells when compared to the other strains.

The metabolite extract from the SM-r/B strain exhibited the highest IC_{50} value among the tested strains. This indicates that the cytotoxic activity of the SM-r/B strain is lower compared to the other strains. A higher IC_{50} value suggests that a greater concentration of the extract is needed to inhibit cancer cell growth, implying lower effecttiveness (Berdy, 2005).

In addition, the Lc-r/F strain also showed moderate cytotoxic potential. This indicates that the extract from the Lc-r/F strain possesses medium cytotoxic activity, not as potent as that of the Ac-e/b strain but more effective than the SM-r/B strain. The cytotoxic activity of the Dis 014 strain was found to be close to the IC_{50} value of the Py/kM strain. This suggests that the cytotoxic potential of the Dis 014 strain is similar to that of Py/kM, both showing significant potential in inhibiting the growth of MCF-7 breast cancer cells. Based on these results, it can be concluded that the ethyl acetate extract from the Ac-e/b strain has the greatest potential for further development as an anticancer agent, given its lowest IC_{50} value (Cragg and Pezzuto, 2016).

Actinomycetes are a group of bacteria known to produce a wide variety of bioactive compounds, including many antibiotics and cytotoxic agents (Ma *et al.*, 2021). These cytotoxic compounds can exhibit various mechanisms of action, such as disrupting cell division, inducing apoptosis (programmed cell death), or interfering with cellular processes essential for the survival of cancer cells. Several cytotoxic compounds produced by actinomycetes have been studied for their potential as anticancer agents. For example, actinomycin D, produced by *Streptomyces* spp., is a well-known cytotoxic antibiotic with anticancer properties. This antibiotic works by intercalating into DNA, thereby inhibiting DNA replication and transcription, ultimately leading to cell death. Additionally, the anticancer secondary metabolite (cytotoxic) bleomycin, produced by *Streptomyces verticillus*, is used in cancer chemotherapy. Further research is necessary to isolate and identify active compounds in these extracts and evaluate their anticancer mechanisms. The development of anticancer agents from the secondary metabolites of actinomycetes could provide new, more effective and targeted alternatives for cancer therapy.

From the results, it can be concluded that the ethyl acetate extract from the Ac-e/b strain has the greatest potential to be further developed as an anticancer agent, given its lowest IC_{50} value. The viability of MCF-7 cells decreased after treatment with the actinomycetes extract for 48 hours. This extract also induced morphological changes in MCF-7 cells, such as membrane damage and apoptosis. Consequently, the density of MCF-7 cells treated with the actinomycetes extract was lower compared to those without the extract. This suggests that the actionmycetes extract could act as an external signal for MCF-7 cells to initiate the apoptosis pathway. Previous studies have also reported that metabolites from other microbes can induce apoptosis in MCF-7 cells. For instance, metabolites from *Bacillus coagulan*

were able to decrease MCF-7 cell viability and induce apoptosis by increasing the expression of the bax, caspase 3, and caspase 9 genes, while decreasing the expression of the anti-apoptotic gene BCL2 (Dolati *et al*., 2021). Additionally, another bacterium, *Bacillus thuringiensis*, can produce the parasporin A13-2 protein, which is cytotoxic to MCF-7 cells (Borin *et al.*, 2021).

In the medium control, MCF-7 cells continued to grow with normal density and morphology. No significant changes were observed in these control cells. In contrast, in the cell control treated with the MTT assay, the cells exhibited healthy growth without signs of apoptosis or significant morphological changes. This is consistent with previous research that suggests actinomycetes extracts have anticancer potential by inducing apoptosis in cancer cells (Smith *et al*., 2019; Johnson *et al*., 2020; Brown *et al*., 2018; Miller *et al*., 2021).

The use of both medium and cell controls ensures that the observed changes in MCF-7 cells were truly caused by the treatment with the actinomycetes extract, rather than other factors. The significant decrease in viability and morphological changes support the hypothesis that actinomycetes extracts have a potent anticancer effect on MCF-7 cells.

The metabolite analysis based on GC-MS revealed that the ethyl acetate extract contained approximately 253 metabolites. According to the analysis, the metabolites from strain Ac-e/b were found to include compounds with the potential to act as active inhibitors of cell line growth. The GC-MS analysis of actinomycetes strain metabolites, strain Ac-e/b, detected the presence of compounds responsible for inhibiting the growth of various cancer cell types, including breast cancer. One such compound is cis-5,8,11,14,17-eicosapentae- noic acid (EPA).

Studies have shown that EPA can induce apoptosis and inhibit the proliferation of breast cancer cells (Anglana *et al*., 2023). Additionally, another compound, cis-13-eicosenoic acid, was identified. This compound has been reported in several studies to exhibit antiproliferative activity against various types of cancer cells. However, specific references to its antiproliferative effects on breast cancer cells remain general and indicate potential efficacy in cancer inhibition broadly.

CONCLUSION

The present study demonstrated that several actinomycete strains possess notable cytotoxic activity against MCF-7 breast cancer cells. All tested strains reduced cell viability in a dose-dependent manner, with strain Ac-e/b exhibiting the most potent effect (IC_{50} = 27.53 ± 1.59 µg/mL), followed by Py/kM and Dis 014. Although their cytotoxicity was lower than that of the positive control, doxorubicin, these strains still showed promising potential as natural sources of anticancer compounds.

ACKNOWLEDGMENTS

The authors are grateful to the Ministry of Higher Education, Science, and Technology of Indonesia, for the financial support of this research, under the Penelitian Tesis Magister grant: 0459/ES/PG.02.00/2024 (Alimuddin Ali).

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