International Journal of Agriculture and Biosciences 2024 13(4): 574-581.
https://doi.org/10.47278/journal.ijab/2024.160

RESEARCH ARTICLE
eISSN: 2306-3599; pISSN: 2305-6622

Antibacterial, Antiaging, and Antiangiogenic Activity of *Streptomyces* sp. SAE4034 Extract from Mangrove Sediment

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ABSTRACT

Streptomyces sp. SAE4034 was isolated from the mangrove rhizosphere in Segara Anakan, Cilacap, Indonesia. It is potentially producing bioactive compounds. The bioactive content was studied for their antibacterial and antiaging activities as well as their potential as antiangiogenic agents. Antibacterial assay was conducted against *Klebsiella pneumoniae* and the acne-causing bacteria *Propionibacterium acnes*. Antiaging testing was done by administering crude extract to *Saccharomyces cerevisiae* cells experiencing oxidative stress due to exposed to 3mM peroxide solution. The antiangiogenic test was assessed through its ability to inhibit the formation of blood vessels in embryonated eggs. The results showed that the *Streptomyces* sp. SAE4034 crude extract contains peptides, polyphenols, polyketides, flavonoids, terpenoids, and alkaloids. The crude extract inhibited *K. pneumoniae* with an average inhibition zone diameter of 16.25mm and a MIC value of 128µg/mL. The inhibitory mechanism against pathogenic bacteria is due to leakage of nucleic acids and proteins, which indicates damage to the *K. pneumoniae* cell wall. Against *P. acnes*, the crude extract produced higher inhibition capability than the positive control; as shown inhibition zone diameter of 44.0850mm, and the MIC value of 32µg/mL. The crude extract prolonged the life span of *S. cerevisiae* cells which experienced oxidative stress. The potential of antiangiogenic substances is proven by significantly reducing the angiogenic process in the chicken embryos development, with morphometric values of 50µg/mL (mean 13.57%) and 100µg/mL (mean 10.93%). *Streptomyces* sp. SAE4034 has the potential to be developed as a source of antibiotics, antiaging, and antitumor compounds.

Keywords: *Streptomyces* sp. SAE4034, Antibacterial, Antiaging, Antiangiogenic, Inhibitory mechanism

Article History
Article # 24-730
Received: 30-Jul-24
Revised: 07-Sep-24
Accepted: 10-Sep-24
Online First: 28-Sep-24

INTRODUCTION

The mangrove environment is an area that provides excellent opportunities for exploration as a potential source of Actinomycetes, especially streptomyces which are as sources of bioactive compounds (Hong et al., 2009; Law et al., 2020). Bioactive compounds produced by Streptomyces group, are known to have many advantages, including antibacterial, antifungal, antiviral, antioxidant, and antiangiogenic properties (Jakubiec-Krzesniak et al., 2018; Mojicevic et al., 2020; Rammali et al., 2022). Actinobacteria isolated from mangrove environment have unknown potential to produce secondary metabolite (Hu et al., 2021). Streptomyces genera are the most important actinomycetes that produce important metabolites (Hegazy et al., 2023).

Several *Streptomyces* isolates were isolated from the rhizosphere of *Rhizophora apiculata* in Segara Anakan, Cilacap. One of which is *Streptomyces* sp. SAE4034, which was collected at the mangrove area in the eastern part of the lagoon (Ryandini et al., 2018). Preliminary test, the crude extract of *Streptomyces* sp. SAE4034 inhibits the growth of MDR bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus* sp., and

Cite this Article as: Ryandini D, Ma'arif S, Hidayat A, Putri AR, Kusharyati DF, Pratiwi M, Wiraswati SM, Fitriadi R and Oedjijono, 2024. Antibacterial, antiaging, and antiangiogenic activity of *Streptomyces* sp. SAE4034 extract from mangrove sediment. International Journal of Agriculture and Biosciences 13(4): 574-581. https://doi.org/10.47278/journal.ijab/2024.160

A Publication of Unique Scientific Publishers

Klebsiella pneumonia (Ryandini et al., 2018). Nevertheless, the capability of *Streptomyces* sp. SAE4034 crude extract on inhibiton against non-MDR pathogenic bacteria and other bacteria, such as *K. pneumoniae* and *Propionibacterium acnes* was not known.

Klebsiella pneumonia is a nosocomial pathogenic Gram-negative bacterium disturbing the hospital environment. This bacterium causes several infections, including sepsis, urinary tract infections (UTI), bacteremia, meningitis, pyogenic liver abscesses, and lower respiratory tract infections (Egbe et al., 2011; Paczosa & Mecsas, 2016). Lower respiratory tract infection (LRTI) is an infection that occurs in the respiratory organs below the epiglottis, for example, pneumonia, bronchitis, and tuberculosis. LRTI is belong to ten desease that cause the most deaths in Indonesia based on the 2018 GBD comparison (CDC, 2020). The inhibition mechanism of *Streptomyces* sp. SAE4304 against *K. pneumonia* and bioactive compounds produced are still unknown. According to Nurkanto et al. (2010), the mechanism of growth inhibition can be observed through cell leak testing by measuring the OD value of protein or nucleic acid at λ 260 and 280nm.

Apart from nosocomial infections by *K. pneumoniae*, one of the serious problems in skin disorders is acne vulgaris, which is a condition when the skin pores are blocked by fat due to the high activity of the oil glands. *P. acnes* bacteria are the main commensal on human skin and contribute to most skin microbiota (Nazipi et al., 2017). Skin conditions worsen when bacteria infection causes acne until it becomes inflamed and fester (Rudiyat et al., 2020). Aging is a natural process characterized by a decline in tissue function or death. Aging can be accelerated by several factors, one of which is high levels of free radicals. Free radicals can be suppressed by adding exogenous antioxidants to the body through drinks, food, and beauty products with antiaging properties. *Streptomyces* sp. SAE4034 can produce antioxidant compounds (Kurniawati et al., 2023), but its ability as an antiaging agent is not yet known.

Antitumor activity can be started with antiangiogenic testing. Analysis of antiangiogenic activity using the chorioallantois membrane (CAM) test of embryonated chicken eggs. The CAM assay has been used for many studies on angiogenesis, tumor cell invasion, and metastasis (Hamid et al., 2018). This test depicts the branching of blood vessels in tumor cells to increase blood supply and ensure their unusual growth. Antiangiogenic substances suppress blood vessel sprouting, preventing tumor growth indefinitely (Usha et al., 2010). The development of bioactive screens from actinomycetes is a challenge.

This article reports a study of the class of antibacterial compounds produced by *Streptomyces* sp. SAE4034 and its mechanism of inhibition against *K. pneumoniae* and its inhibition against *P. acnes*. The study also discusses the benefits of bioactive compounds produced by *Streptomyces* sp. SAE4034 in inhibiting aging (antiaging) and antiangiogenic activity.

MATERIALS & METHODS

Ethical Clearance

This research did not use animals so we did not register for ethical clearance.

Isolate Subculture

Isolate *Streptomyces* sp. SAE4034 was grown on starch casein nitrate agar (SCNA) medium and then incubated for 5-7 days at room temperature. *K. pneumoniae* and *P. acnes* isolates were subcultured on Nutrient Agar (NA) medium, and *S. cerevisiae* was subcultured on Potato Dextrose Agar (PDA) medium, incubated at room temperature for 24 hours.

Production and Extraction of Bioactive Compounds *Streptomyces* sp. SAE4034

Culture of *Streptomyces* sp. SAE4034, aged 7 x 24 hours, was made into an inoculum plug. A total of 20 plugs were inoculated into 200mL of starch casein nitrate broth (SCNB) medium and then incubated for 28 days with stationary fermentation at room temperature. The fermentation results were then filtered using Whatman paper no. 42 to separate biomass and fermentation filtrate. The filtrate was collected and then extracted using ethyl acetate with a ratio of 1:1 and shaken for 60minutes. The two layers obtained were then separated using a separating funnel, and the extract containing bioactive compounds was collected in a separate container. Then, the extract from ethyl acetate was evaporated using a rotary evaporator at a temperature <70℃ for two hours to obtain a thick crude extract.

Determination of Antibacterial Compound Groups using Thin-layer Chromatography (TLC) and Phytochemical Methods

A TLC aluminum silica gel 60 F254 plate was prepared, and 5µL of crude extract was spotted on the starting line. The plate was then immersed in an eluent containing a mixture of hexane and ethyl acetate (3:1). After eluting, the plate was dried at 80℃ for 2 hours and then observed under UV light (λ 366nm) in a UV cabinet. The spots formed were observed, and the spot Rf value was calculated.

The phytochemical method was carried out to determine the class of compounds contained in the spots formed. The flavonoid compound group detected with cytoborate reagent is characterized by the formation of yellow or greenish-yellow spots under 366nm UV light. The alkaloid group of compounds is detected using Dragendorf's reagent. A positive reaction is characterized by a brown or orange-brown and orange-red color with a yellow-to-gray background at UV 366nm. The polyphenol group of compounds is detected using FeCl3 spray reagent and is characterized by the formation of gray, green, and blue spots in visible light. The peptide compound group was detected using ninhydrin spray reagent, which was dried in an oven at 105℃. The presence of peptides was detected by the formation of a purple color on the spots. The carbonyl group

(aldehyde/ketone) was detected using the 2,4 DNPH spray agent and then dried using an oven at 105℃. The formation of yellow, orange, and red colors identified the presence of the compound.

Antibacterial, MIC, and Bioautography Tests of Antibacterial Compounds of *Streptomyces* sp. SAE4034 against *K. pneumoniae*

K. pneumoniae was inoculated on Mueller Hinton Agar (MHA) medium. A paper disc with a diameter of 6mm was dripped with 20µL of crude extract and then placed on lawn of bacteria. The culture was incubated for 1 x 24 hours at 37℃. Gentamicin was a positive control, and distilled water was a negative control. The diameter of the clear zone formed was measured. The effectiveness of inhibition was calculated using the formula:

$$E = \left(\frac{D}{Da}\right) \times 100\%$$

Note: E = inhibitory effectiveness (%), D = diameter of the extract inhibition zone (mm), Da = diameter of the positive control inhibition zone (mm).

The MIC test against *K. pneumoniae* begins by diluting the crude extract using distilled water to obtain concentrations of 0, 25, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128µg/mL. The 100µL extract in distilled water and 100µL of *K. pneumoniae* suspension were put into a 96-well plate (Biologix). The negative control was 100µL of the test bacterial suspension and 100µL of distilled water. In comparison, the positive control was added with 100µL of gentamicin to 100µL of the test bacterial suspension. The plate was incubated at 37℃ for 1 x 24hrs. MIC values were based on the lowest apparent concentration indicating no growth of *K. pneumoniae*.

A bioautography test determined the class of compounds that inhibit *K. pneumoniae*. The TLC plate that has been eluted was placed in a Petri dish containing cotton wool dripped with sterile distilled water to provide moist conditions. *K. pneumoniae* was sprayed on a TLC plate and then incubated at 37℃ for 1 x 24 hours. After incubation, the TLC plate was dripped with 2,3,5-triphenyl tetrazolium chloride (TTC) to visualize the zone of inhibited bacterial growth. The plate was re-incubated at 37℃ for 1 x 24 hours and then observed for the inhibition zone formed, namely the area that was not colored red or red color that is more transparent than its surroundings.

The inhibition mechanism test of the antibacterial compound of Streptomyces sp. SAE4034 against *K. pneumoniae*

K. pneumoniae was cultured in 10mL of NB, to which 128µg/mL of crude extract was added and incubated for 1 x 24 hrs. Sample without crude extract as control. After incubation, 3mL of the culture and crude extract mixture was centrifuged at 3011 x G for 15min. Then, the supernatant was collected and diluted 20 times to determine its conductivity using a conductivity meter. Conductivity measurements for measuring cell membrane permeability. The rate of change in conductivity is calculated using the formula:

$$R\ (\%) = \frac{Rs - Rc}{Rc} \times 100\%$$

Note: R = rate of change in conductivity (%), Rs = conductivity of the treatment, Rc = conductivity of the control.

Protein and nucleic acid leakage are determined by analyzing OD values at 260 and 280nm wavelengths. *K. pneumoniae* was grown in 10mL NB medium for 1 x 24 hours. The culture suspension was added with 0.5mL of Tween 80 and centrifuged at a speed of 2305 x G for 20 minutes at a temperature of 4℃. The filtrate was discarded, and the pellet was resuspended with 0.1% phosphate buffer pH 7.0 twice, then suspended in 10mL of phosphate buffer pH 7.0. Crude extract of the antibacterial compound *Streptomyces* sp. SAE4034 with 128 and 256µg/mL concentrations were added to the test bacterial suspension. Bacterial suspension without adding extract was used as a control treatment. The liquid culture was then incubated for 1 x 24 hours. After incubation, the suspension was centrifuged at 2305 x G for 15min at 4℃. The supernatant liquid was taken, and its absorbance was measured at 260 and 280nm wavelengths using a UV/VIS spectrophotometer.

Antibacterial Test and MIC of Antibacterial Compounds of *Streptomyces* sp. SAE4034 against *P. acnes*

P. acnes were inoculated on Mueller Hinton Agar (MHA) medium. A paper disc with a diameter of 6mm was dripped with 20 µL of crude extract and then placed on surface of a spread of test bacteria. The culture was incubated for 1 x 24 hours at 37℃, with tetracycline as the positive control and distilled water as the negative control.

The *P. acnes* MIC test was conducted by mixing 800µL of NB medium, 100µL of crude extract with different concentrations in distilled water, and 100µL of *P. acnes* liquid culture in a test tube. Tetracycline was used as the positive control, and distilled water was used as the negative control. All tubes were incubated for 1x24 hours at 37℃. The MIC value was determined in a test tube with the lowest concentration which appeared clear.

Antiaging Test of Crude Extract of *Streptomyces* sp. SAE4034 against *Saccharomyces cerevisiae* Yeast Cells Experiencing Oxidative Stress

The extract was diluted with distilled water at 500, 1000, 1500, and 2000ppm concentrations. Each extract with different concentrations was added to 3mL of PDB medium. *S. cerevisiae* isolate was inoculated into each extract concentration treatment. The 3mM H_2O_2 solution was added as much as 1mL to trigger oxidative stress. *S. cerevisiae* grown in PDB medium with 3% glucose without extract was used as a negative control, while *S. cerevisiae* cultured in PDB containing 0.5% glucose without extract was used as a positive control.

Each culture was incubated for 11 days, and the aging test was carried out with a spot test on days 7 and 11 of the incubation period. Each culture was adjusted to OD = 1 and serially diluted (up to 10^{-4}). A total of 2µL of each aqueous suspension was then inoculated pointwise into PDA medium and incubated for 1-3 days at room temperature. The spot test results the growing yeast then the colony diameter was measured.

Antiangiogenic Test of Crude Extract of *Streptomyces* sp. SAE4034

The antiangiogenic assay was conducted based on modification of Deryugina & Quigley (2008), Marinaccio & Ribatti (2015), and Saravanan et al. (2020). Six-day-old chicken embryos were placed by candling, and the blunt end of the egg was marked for the injection position. Crude extract of *Streptomyces* sp. SAE4034 with 50 and 100µg/mL concentrations was injected into albumin. Celecoxib was used for positive control and negative control without treatment. Eggs were incubated for two days at 37℃ in an incubator with 55-60% humidity. After incubation, the eggs were carefully broken in half. Morphometric values of blood vessel formation in chicken embryos were determined using ImageScope 12.4.3 software and compared with positive control (Celecoxib) and negative control (no treatment). A substance is said to have antiangiogenic activity if the index is lower than the positive control.

Statistical Analysis

Data on bacterial growth inhibition, antiaging activity and antiangiogenic activity were analyzed using ANOVA at a 95% confidence level, while other data were analyzed descriptively.

RESULTS & DISCUSSION

Determination of Bioactive Compounds of *Streptomyces* sp. SAE4034

The TLC test results showed six spots with Rf values from the lowest to the highest, namely 0.02, 0.25, 0.49, 0.63, 0.79 and 0.95. The results of the phytochemical analysis showed the presence of alkaloid, polyphenol, flavonoid, and peptide compounds. Previous studies also reported these compounds and their properties (Rachma & Saptawati, 2020; Syarifuddin & Sulistyani, 2019). The alkaloid compound class was marked with an orange color at Rf 0.95, the polyphenol compound class was marked with a gray color at Rf 0.02, 0.25, 0.49, and 0.95, the flavonoid compound class was marked with a yellow color at Rf 0 .25 and 0.95, and the peptide compound group was characterized by a purple color at Rf 0.63 and 0.79. The results of the phytochemical test using 2,4-DNPH reagent showed a negative interpretation for carbonyls (aldehydes/ketones) because no orange color formed on the plate spot after spraying with 2,4-DNPH reagent.

Inhibition of *K. pneumoniae* by Crude Extract of *Streptomyces* sp. SAE4034

The inhibition test results showed that the crude extract of *Streptomyces* sp. SAE4034 has inhibitory activity against the growth of *K. pneumoniae*. The diameter of the inhibition zone formed was 16.25±2.7mm (Fig. 1). The effectiveness of the inhibition zone of crude extract compounds against *K. pneumoniae* was 61.32%. According to research by Azis & Cahyadi (2020), the inhibitory efficacy of 20.3 – 21.2% of the extracted compound against the test pathogen is considered a robust inhibitory response. So, the inhibition of the antibacterial compound of ethyl acetate crude extract of *Streptomyces* sp. SAE4034 against pathogenic bacteria was included as a strong response.

The MIC test results showed that the crude extract inhibited the growth of *K. pneumoniae*, starting at a concentration of 128µg/mL (Fig. 1). Tangjitjaroenkun (2018) obtained the MIC results from the ethyl acetate extract of *Streptomyces omiyaensis* SCH2 isolated from mangrove sediments against *K. pneumoniae* was 1mg/mL. Based on this research, the ethyl acetate crude extract of *Streptomyces* sp. SAE4034 has a higher ability to inhibit the growth of *K. pneumoniae*.

The results of the bioautography test showed the inhibition of *K. pneumoniae* by several spots that formed. Inhibition is characterized by the presence of an area that is not colored red or is red and thinner compared to the surroundings (Czernicka et al., 2019). The results showed that there were zones with a red color that were more transparent than the surroundings, namely in spots with Rf 0.63, 0.79, and 0.95. Based on these results, spot fractions at Rf 0.63, 0.79, and 0.95 have antibacterial ability against *K. pneumoniae*. Peptide compounds, alkaloids, polyphenols, and flavonoids were antibacterial against *K. pneumoniae*. Browne et al. (2020) revealed that the peptide compound class is a compound that is widely found in nature. Several peptide compounds have antimicrobial capabilities, and it is known that ten commercially available antimicrobials are antimicrobials from the peptide class. Amber et al. (2018) and Rita et al. (2019) explained that the phenol, polyphenol, and alkaloid groups can have antimicrobial abilities but different levels depending on the total content of the extract compounds. Othman et al. (2019) explained that the performance of extract treatment containing alkaloid and polyphenolic compounds with novobiocin provided an excellent synergistic effect in fighting *K. pneumoniae*.

Fig. 1: The growth inhibition zone of *K. pneumoniae* by crude extract of *Streptomyces* sp. SAE4034 (left) and MIC test results (right): Description: line 1: repetition 1, line 2: repetition 2, line 3: repetition. Left to right columns are crude extract concentrations of 256, 128, 64, 32, 16, 8, 4, 2, 1, and 0.5µg/mL, positive control and negative control

The inhibition mechanisms of the crude extract of *Streptomyces* sp. SAE4034 were analyzed by testing cell membrane permeability, protein, and nucleic acid leakage. The test results showed that the conductivity values in cultures treated with extract concentrations of 128 and 256µg/mL, respectively, were 154.37±1.5 and 156.33±1.3 µS. The following two values were calculated by the percentage rate of change in conductivity, and it was found that the percent change in conductivity for extract concentrations of 128 and 256µg/mL was 16.15% and 17.63%. The increase in conductivity indicated that *K. pneumoniae* experienced damage to the cell membrane and cell wall due to adding the extract compound. According to Lin et al. (2018), damage to the cell membrane can cause electrolyte leaks and increase the cell's electrical conductivity. The results of cell leak test observations showed that there was leakage of protein and nucleic acid from *K. pneumoniae*. The absorbance value of the crude extract concentration treatment of 128 and 256µg/mL was 0.491 at λ 260nm and 0.342 for λ 280nm. Increased conductivity, protein, and nucleic acid leakage indicated an inhibitory mechanism of *Streptomyces* sp. SAE4034 ethyl acetate crude extract against *K. pneumoniae,* that was increased cell membrane permeability and which induced cell leakage.

Antibacterial Activity of Crude Extract of *Streptomyces* sp. SAE4034 against *P. acnes*

The antibacterial activity of crude extract of *Streptomyces* sp SAE4034 against *P. acnes* was tested using the diffusion method (Kirby-Bauer) and showed very high inhibition. The larger the diameter of the inhibition zone formed, the stronger the inhibitory activity of the antibacterial compound (Sastry & Barth, 2018). A crude extract concentration of 100% produces an average inhibitory zone diameter of 44.0850mm, and a concentration of 50% produces an average inhibitory zone diameter of 40.185mm (Fig. 2). The effect of different extract concentration was not significant ($p>0.05$), although according to Davis & Stout (1971) the inhibitory effect was very strong because the inhibition zone diameter is >20mm. The diameter of the resulting inhibition zone was higher than the diameter of the inhibition zone of the positive control tetracycline (22.2438mm). Tetracycline is categorized as potent in inhibiting the growth of *P. acnes*. This diameter was larger than the research results of Nita et al. (2018), namely 18.88mm. Dahal et al. (2017) showed that isolates of *Streptomyces* spp. able to inhibit the growth of *P. acnes*, with an inhibition zone between 20.30±1.20 and 28.70±0.70. Lee & Song (2018) showed inhibition of *Streptomyces* spp. against *P. acnes* can reach the highest diameter, 34.3mm. The inhibition of crude extract against *P. acnes* remained consistent until >48 hours of incubation. Inhibitory activity >48 hours indicates that the crude extract can be a stable antibacterial agent (Ryandini et al., 2018).

The MIC test results showed that a concentration of 32µg/mL was the lowest concentration that inhibited the growth of *P. acnes*. According to Jha et al. (2022), the MIC value for *P. acnes* is 31.25µg/mL. Ochsendorf (2010) also stated that secondary metabolite compounds of *S. aureofaciens* had a MIC value of 32µg/mL against *P. acnes*. The MIC value is included in the strong category based on the Clinical & Laboratory Standard Institute. Zhu et al. (2019) stated that *P. acnes* bacteria are susceptible to tetracycline administration. Tetracycline can suppress the growth of Gram-positive bacteria, such as *P. acnes*, by interfering with protein synthesis. Based on the research results, *Streptomyces* sp. SAE4034 has the opportunity to be a source of antibacterial compounds that are high in strength and stable against the growth of *P. acnes*.

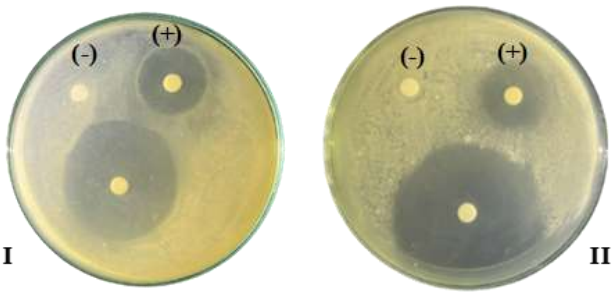

Fig. 2: Inhibition of *P. acnes* by crude extract of *Streptomyces* sp. SAE4034; Description: (I) Extract concentration 50%; (II) 100% Extract Concentration

The Antiaging Activity of Crude Extract of *Streptomyces* sp. SAE4034 on *S. cerevisiae*

Analysis of variance (Anova) of extract concentration on yeast cell lifespan was non-significant ($p>0.05$), but descriptively it can be seen that administration of crude extract was able to increase the life span of yeast cells up to 11 days. The results of the tolerance test against oxidative stress due to a 3mM peroxide solution using the spot test showed that the crude extract with various concentrations and dilutions could support yeast cell viability at 7 and 11 days of observation (Fig. 3). The diameter of yeast colonies with crude extract was higher than in the negative control (Fig. 4).

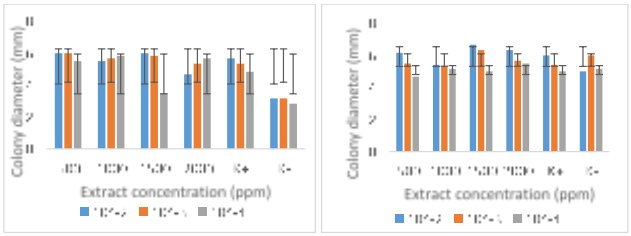

Fig. 3: Diameter of *S. cerevisiae* colonies after antiaging test of crude extract of *Streptomyces* sp. SAE4034 with different concentrations after an incubation period of 7 days (left) and 11 days (right)

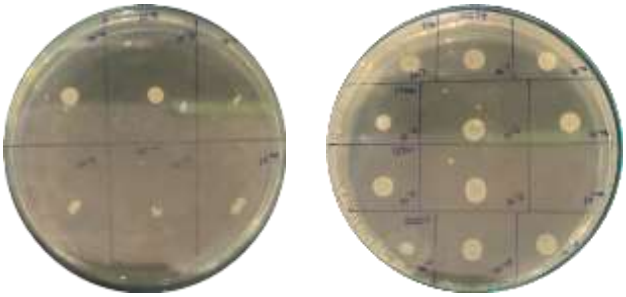

Fig. 4: Seven days of antiaging test results for the crude extract of *Streptomyces* sp. SAE 4034 (right) against *S. cerevisiae* cells with positive control (top left) and negative control (bottom left)

H_2O_2 acts as a free radical that causes oxidative stress, accelerating aging. According to Collin (2019), hydrogen peroxide is included in the Reactive Oxygen Species (ROS) group, which is not dangerous. High amounts of hydrogen peroxide can disrupt cell function and damage biomolecules. When ROS is too high, additional antioxidants are needed to balance cell conditions. These antioxidants will protect cells from damage to function that causes aging. Based on observations, 3mM H_2O_2 stress can still maintain the life span of yeast. An extract concentration of 500ppm can maintain yeast lifespan despite H_2O_2 stress.

Antiangiogenic Activity of Crude Extract of *Streptomyces* sp. SAE4034 against Embryonated Eggs

The analysis results showed significant differences in the strong positive pixel values that identified the formation of blood vessel branching (Table 1). CAM treated with 100µg/mL crude extract showed lower morphometric values (mean= 10.93%, SD=1.34) than 50µg/mL (mean= 13.57%, SD= 1.38). Both concentrations did not significantly affect angiogenesis inhibition compared to the negative control. The negative control had the highest value (mean 29.37%, SD= 1.93), while the positive control had the lowest value (mean 6.21%, SD= 2.52). No significance was found between 100µg/mL and the positive control. The difference in morphological values indicated the possibility of antiangiogenic compounds in the crude extract of *Streptomyces* sp. SAE4034. The visual appearance of the embryos shows that the negative control has blood vessels that are larger, longer, and more branched than the other treatments (Fig. 5).

Table 1: Morphometric value of angiogenesis of chicken egg embryos treated with crude extract of *Streptomyces* sp. SAE4034

Concentration	Average Morphometric Value (%±SD)
Negative control	29.37±1.93c
50µg/mL	13.57±1.38b
100µg/mL	10.93±1.34ab
Positive control	6.21±2.52a

The inhibition mechanism of angiogenesis by crude extract of *Streptomyces* sp. SAE4034 on chicken embryos is still unknown. The mechanism of the angiogenesis inhibition process is comprehensive and varies based on the type of inhibitor compound. However, based on the results of TLC and phytochemical tests, polyphenolic compounds, flavonoids, terpenoids, and polyketides may play a role in inhibiting the development of angiogenesis. Research by Mirossay et al. (2018) and Abbaszadeh et al. (2019) revealed that polyphenolic and flavonoid compounds, such as quercetin and luteolin, can inhibit the phosphorylation of vascular endothelial growth factor (VEGFR) and are also able to inhibit cell proliferation. On the other hand, kaempferol and polyphenols can also

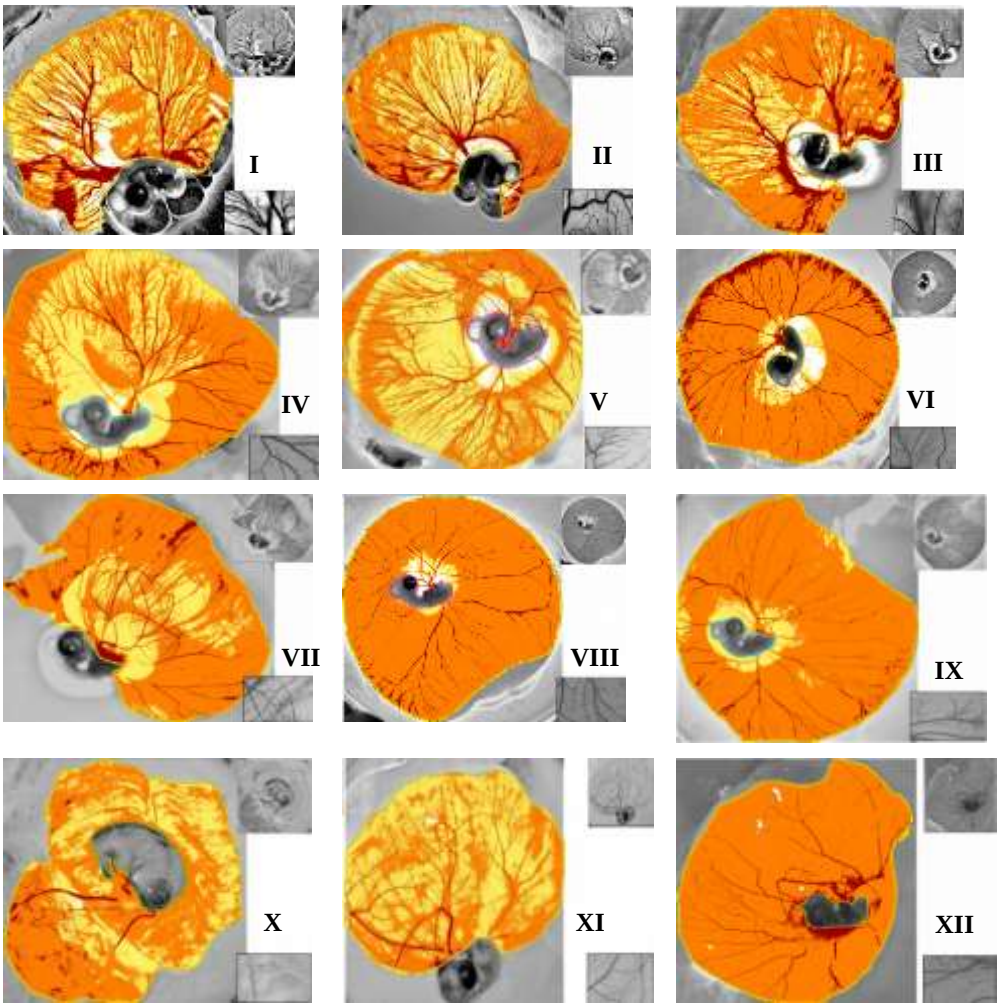

Fig. 5: Image Analysis for Morphological Value Determination of Embryonated Chicken Eggs; Details: (I-III) Image analysis of angiogenesis in embryonated chicken eggs treated with Negative control; (IV-VI) Image analysis of angiogenesis in embryonated chicken eggs treated with 50µg/mL; (VII-IX) Image analysis of angiogenesis in embryonated chicken eggs treated with 100 µg /ml; (X-XII) Image analysis of angiogenesis in embryonated chicken eggs treated with Positive control.

inhibit VEGF secretion. Other compounds can suppress hypoxia-inducible factor 1-alpha (HIF-1α), a protein complex that triggers the synthesis and release of proangiogenic factors in tissue or cell hypoxia conditions. Qi et al. (2020) argue that terpenoids have antiangiogenic properties by suppressing the VEGFR/Akt signaling pathway, inhibiting endothelial cell migration and blood vessel growth in vitro. Polyketides are also known to have a mechanism of inhibiting endothelial cell migration due to decreasing levels of VEGF, making endothelial cells unable to penetrate the basement membrane and attack the stroma of the surrounding tissue, where they form new blood vessels (Yang et al., 2017). However, clinical-stage antiangiogenic drugs that have been approved and implemented have focused mainly on inhibiting growth factors and receptors, such as VEGF and VEGFR signaling (Mirossay et al., 2018). VEGF plays an essential role in the angiogenesis process. These growth factors are specific essential signaling proteins that promote the growth of new blood vessels by inducing vascular permeability and stimulating endothelial cell growth. In addition, VEGF also plays a vital role in fostering epithelial-mesenchymal transition and the proliferation of stem and progenitor cells in tumors (Yang et al., 2017).

In general, the process of inhibiting angiogenesis can occur in 2 ways. The direct way is to suppress vascular endothelial cells' proliferation and migration processes in response to angiogenic proteins, for example (vascular endothelial growth factor) VEGF. An indirect way is to modify the expression of angiogenic proteins and their activity, such as receptor regulation on endothelial cells (Marinaccio & Ribatti, 2015). Inhibition of endogenous angiogenic factors such as bFGF, VEGF, and circulating endothelial progenitor cells, inhibits degradation enzymes (matrix metalloproteinases) responsible for basement membrane degradation, inhibits endothelial cell proliferation, inhibits endothelial cell transfer, and inhibits endothelial cell activation and differentiation. bFGF and VEGF are the main components in the angiogenesis process in wounds and cancer cells (Hamid et al., 2018).

Conclusion

Based on the results obtained, it can be concluded that the crude ethyl acetate extract of *Streptomyces* sp. SAE4034 contains polyphenol, flavonoid, peptide, and alkaloid compounds. Ethyl acetate crude extract of *Streptomyces* sp. SAE4034 can strongly inhibit *K. pneumoniae* through a mechanism that damages cell walls and increases cell membrane permeability, thereby triggering cell leakage. Extreme inhibition also occurred in the growth of *P. acnes*. The crude extract can extend the life span of *S. cerevisiae* yeast cells that experience oxidative stress and reduce the formation of blood vessels in egg embryos. *Streptomyces* sp. SAE4034 has excellent potential to be developed in the industrial, health, and beauty sectors as a source of antibiotics, antiaging, and antitumor compounds. Further studies are needed to determine the types of compounds that play a role, and research using a molecular approach is required so that the gene system that plays a role in antibacterial, antiaging, and antiangiogenic activity is known.

Acknowledgement

The authors are grateful to BLU Unsoed 2021 and 2023 for funding this study.

Competing Interests

The authors declare that they have no competing interests.

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