Formosa Journal of Science and Technology (FJST)
Vol.2, No.12, 2023: 3175-3190

Optimization of Antibacterial Metabolite Production and Antioxidant Activity of Endophytic Fungi Isolate from Kelakai (Stenochlaena palustris)

Harlyanti Muthma'innah Mashar[1*], Teguh Supriyono[2], Suhaera[3], Ismail[4], Dali[5], Muhammad Isrul[6]
[1,2]Nutrition Department, Poltekkes Kemenkes Palangka Raya
[3]Pharmacy Study Program, Institut Kesehatan Mitra Bunda, Batam
[4]Sekolah Tinggi Ilmu Farmasi Makassar
[5]Nursing Department, Poltekkes Kemenkes Kendari
[6]Departement of Pharmacy, Universitas Mandala Waluya Kendari
Corresponding Author: Harlyanti Muthma'innah Mashar
harlyanti@polkesraya.ac.id

A R T I C L E I N F O
Keywords: Kelakai, *Stenochlaena Palustris*, Endophytic Fungi, Antibacterial Activity, DPPH

Received : 05, November
Revised : 10, November
Accepted: 15, December

A B S T R A C T
This research aims to optimize the production of antibacterial metabolites and test the antioxidant activity of endophytic isolates from kelakai (*Stenochlaena palustris*). The research results showed that the optimum growth profile of the isolate showed that exponential growth only started on day 1 and reached a maximum on day 17. Based on pH variations, it shows that pH 7 is the optimum pH in inhibiting bacterial growth, and based on variations in carbon sources, it shows that fructose is a better carbon source than glucose. Antimicrobial activity test using ethyl acetate extract from endophytic fungal fermentate based on differences in pH and carbon source showed antimicrobial activity against *S. aureus* and *E. coli* with the inhibition zone diameters being 13.50 mm and 11.35 mm, respectively.

DOI Prefix: https://doi.org/10.55927/fjst.v2i12.7040
ISSN-E: 2964-6804
https://journal.formosapublisher.org/index.php/fjst

INTRODUCTION

Innovations based on natural ingredients that have potential as antibiotic and antioxidant compounds are being developed in line with the increasing number of types of microorganisms that are resistant to antibiotics (Rumidatul et al., 2021). Research on secondary metabolites obtained from endophytes is the focus of the search for new antibiotic compounds aimed at overcoming these problems and to ward off free radical compounds continues to increase (Aini et al., 2022; Mookherjee et al., 2020; Utami et al., 2023). Microorganisms can cause infectious diseases and various other diseases such as cancer, asthma and cardiovascular disorders (Preuttiporn & Sita, 2019).

Endophytic fungi are fungi that colonize plant tissue as hosts, without causing damage or causing disease in plants (Aini et al., 2022; Gouda et al., 2016; Jia et al., 2016). Endophytic fungi have an effective source of bioactive compounds, have low toxicity, low cost, fast growth, and use on an industrial scale provides minimal impact on the environment (Pandy et al., 2023; Preuttiporn & Sita, 2019).

Various bioactive compounds such as alkaloids, tannins, terpenoids, steroids and phenolic derivatives have potential as mycotoxins, enzymes and antibiotics (Handayani et al., 2018; Pandy et al., 2023). In addition, bioactive compounds from endophytic fungi also show strong antiparasitic and antioxidant activity (Praptiwi et al., 2018; Preuttiporn & Sita, 2019).

Antioxidants obtained naturally are very effective in preventing destructive processes caused by free radicals. Free radicals can cause various diseases including cancer, hypertension, diabetes, neurodegenerative disorders, and others (Dewage et al., 2022; Michalak, 2022; Sharifi-Rad et al., 2020). Synthetic antioxidants have been shown to have toxic effects on the body. So, it is very important to look for new sources of antioxidants and antimicrobial compounds that are safe and easily available locally and come from natural sources (Adeleke & Babalola, 2021; Elfita et al., 2022).

Endophytic fungi isolated from Kelakai (*Stenochlaena palustris*) have antimicrobial activity and contain compounds that have potential as antioxidants. Three endophytic fungi isolated from Kelakai, identified as *Aspergillus* sp., *Paecilomyces* sp., and *Arthrocristula* sp., were tested for antimicrobial activity. Antimicrobial activity showed good inhibition results against *S. aureus* and *E. coli* (Mashar et al., 2023). In order to increase the production of bioactive compounds, optimization of the production of bioactive compounds was carried out on isolates which in previous research showed the best activity, and identified optimal conditions for production.

The production of bioactive compounds in endophytic fungi is influenced by several factors, including medium composition, nutrient availability, lighting, stirring, variations in carbon sources and pH. Optimization of bioactive compound production is carried out to obtain optimum conditions for the growth of endophytic fungi (Rumidatul et al., 2021).

The production of bioactive compounds from endophytic fungi is closely related to the condition of the medium and the length of incubation time. A suitable environment is very supportive of the production of bioactive

compounds from endophytic fungi, so it is important to create optimal environmental conditions for the growth of endophytic fungi (Saleh et al., 2019; Widjajanti et al., 2022; Zhao et al., 2021).

METHODOLOGY

Material

The materials used in this research were endophytic fungal isolates obtained from previous research, aluminum foil, distilled water, blank disks, hydrochloric acid, sodium hydroxide, 0.9% physiological NaCl, blue tip, yellow tip, 70% ethanol, ethyl acetate, DMSO , sodium hypochlorite (NaOCl), filter paper, fructose, Nutrient Agar (NA), Nutrient Broth (NB), Potato Dextrose Agar (PDA), Potato Dextrose Yeast (PDY), Methanol, 2,2 Diphenyl 1 picrylhydrazyl (DPPH), chloramphenicol, Tetracycline, cotton, tissue, *Staphylococcus aureus*, and *Escherichia coli*.

Making Nutrient Agar Media

A total of 23 grams of NA powder was dissolved in 1000 mL of distilled water then heated. After that, measure the pH of the media and add HCl or NaOH to obtain a pH of 7. The media is then sterilized.

Making Potato Dextrose Yeast Media

A total of 19.8 g of GDP was dissolved in 750 ml of distilled water and then heated. Once dissolved, add 3 grams of yeast extract and heat. Measure the pH of the media and add HCl or NaOH to obtain a pH of 6, then divide it into 15 glass bottles and then sterilize.

Making Potato Dextrose Yeast Media pH 4, 7 and 8

A total of 50 mL of PDY was put into a glass bottle. Measure the pH of the media and add HCl or NaOH to obtain a pH of 4, 7 and 8 then sterilize.

Making Potato Dextrose Yeast + Glucose Media pH 4, 7 and 8

A total of 50 mL of PDY was put into a glass bottle then add 1 gram of glucose into each0020bottle. Measure the pH of the media and add HCl or NaOH to obtain a pH of 4, 7 and 8 then sterilize.

Making Potato Dextrose Yeast + Sucrose Media pH 4, 7 and 8

A total of 50 mL of PDY was put into a glass bottle then add 1 gram of fructose into each bottle. Measure the pH of the media and add HCl or NaOH to obtain a pH of 4, 7 and 8 then sterilize.

Making Starter Cultures for Endophytic Fungi Isolates

A starter culture of endophytic fungal isolates is made by inserting 1 active endophytic fungal isolate which has been incubated for 5-7 days into a glass bottle containing 50 mL of PDY media. The starter culture is then incubated for 3 days.

Making Bacterial Suspensions

Staphylococcus aureus and *Escherichia coli* bacterial inoculum was obtained by inoculating 1 ose pure colonies. The inoculum was diluted using 0.9% physiological NaCl to the equivalent of 0.5 McFarland standard.

Fermentation of Endophytic Fungi Isolates

To obtain secondary metabolites, the active isolate was fermented by inoculating 1 ml of starter culture into a glass bottle containing 50 ml of PDY media. Fermentation was carried out for 14 days at room temperature.

Determination Growth Profile of Active Endophytic Fungi Isolates

In the fermentation process, fermentate is taken and fungal mycelia are harvested every 24 hours to obtain the growth profile of endophytic fungi. The growth profile of endophytic fungi was created based on the increase in the dry cell weight of the fungal mycelia and testing the activity of the fermentation fluid.

Testing Fermentate Activity from Growth Profile

The fermentation liquid obtained from fermentation every day is then tested for antimicrobial activity against approximately 20 µL of *Staphylococcus aureus* and *Escherichia coli* bacteria. Then incubated at room temperature for 1 day at 37˚C. Antimicrobial activity was measured based on the inhibition zone formed around the endophytic fungus.

Optimization of Fermentation Conditions for Active Endophytic Fungi Isolates with Variations in pH and Medium Substrates in the Production of Fermentation Secondary Metabolites

A total of 1 mL of starter culture was inoculated into a glass fermentation bottle which previously contained 50 mL of PDY media, PDY+Glucose media, PDY+Sucrose media respectively with pH variations of 4, 7 and 8. Fermentation was carried out according to the optimum time for the growth of the active isolate. previously obtained.

Fermentation Activity Testing from Optimization of Fermentation Conditions

The fermentation liquid obtained from the Optimization of Fermentation Conditions was then tested for antimicrobial activity against approximately 20 µL of *Staphylococcus aureus* and *Escherichia coli* bacteria. Then incubated at room temperature for 1 day at 37˚C. Antimicrobial activity was measured based on the inhibition zone formed around the endophytic fungus.

Fermentation Production of Active Isolates on a Large Scale

As much 5 mL of starter culture was inoculated into an Erlenmeyer flask containing 250 mL of the Fermentation Condition Optimization media obtained previously.

Fermentation Media Extraction

After the fermentation process ends, the fermentation medium is separated from the fungal mycelia by filtering it using filter paper. The fermentation medium was fractionated with ethyl acetate using a ratio of 1:1. The fermentation medium and ethyl acetate were put into a separating funnel, then shaken gently for approximately 5 minutes. Next, let it sit to separate the media and ethyl acetate. The ethyl acetate fraction was separated and evaporated to obtain a thick extract. This process is carried out until a clear ethyl acetate fraction is obtained.

Test Activity of Ethyl Acetate Extract Active Isolate of Endophytic Fungi

The antimicrobial activity test of ethyl acetate extract from endophytic fungal samples was carried out on *Staphylococcus aureus* and *Escherichia coli* using the disk diffusion method. The paper disc is soaked in the sample with a concentration of 100% (extract in DMSO solvent). The paper disc was then placed in NA media containing bacterial culture, then incubated at room temperature for 1 day. The diameter of the inhibition zone formed was observed and measured. In this test, a control is also used to compare the test results against the sample. The controls used were 3 µl DMSO (negative control), and 3 µl Tetracycline 1.2 mg/ml (positive control). Each test uses three replications, and the mean value ± standard deviation is also described.

Antioxidant Activity Test

Antioxidant activity was determined by the DPPH method using methanol as a blank. The extract was diluted with methanol and divided into several concentration variations, namely 10, 20, 30, 40, and 50 µg/mL. To each solution, 28 µg/mL DPPH solution was added with a ratio of 1:5. Incubated ondark room for 30 minutes. After that, the absorbance was measured at a wavelength of 517 nm using UV-vis spectrophotometry (Gurgel et al., 2023; Rammali et al., 2022).

RESULTS

Determination Growth Profile of Active Endophytic Fungi Isolates

The growth of endophytic fungal mycelia that has been fermented for 21 days is shown in Figure 1. These results show that endophytic fungi from kelakai can grow well on PDY media, so it is hoped that they can produce good bioactive compounds too. The curve graph shows that in the initial stage of growth it is still in the adaptation phase so that exponential growth only begins on day 1 and reaches a maximum on day 17. Static conditions were seen on days 3 to 16, and began to decline on day 18. The growth curve was measured by measuring the weight of the mycelia during the fermentation process.

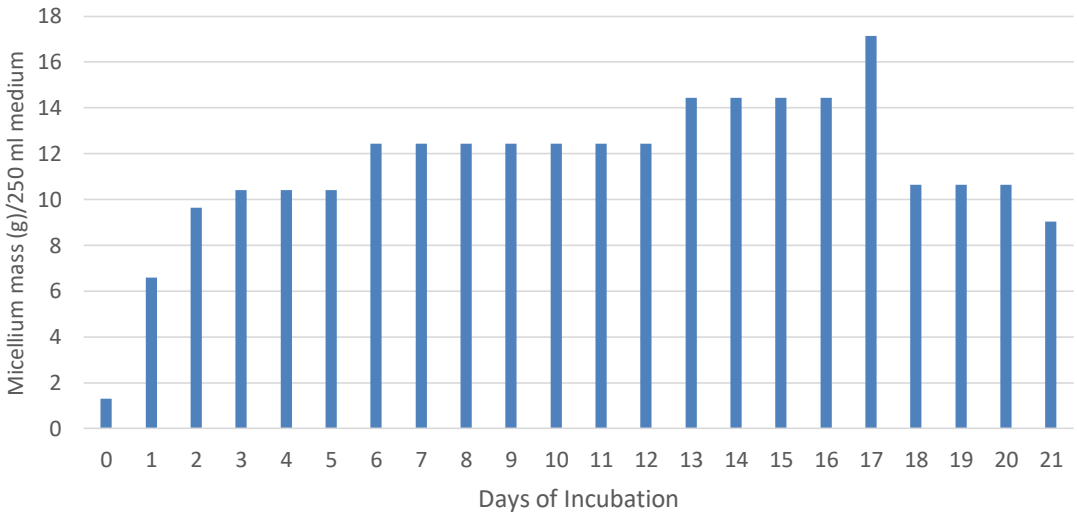

Figure 1. Growth curve of endophytic fungi from kelakai

Testing Fermentate Activity from Growth Profile

Table 1. Fermentate Antimicrobial Activity Test Results

Days to	Inhibition zone diameter (mm)	
	S. aureus	*E. coli*
1	5.14	4.34
2	5.38	4.87
3	6.95	5.81
4	6.95	5.85
5	6.95	5.85
6	8.15	7.33
7	8.15	7.03
8	8.15	7.21
9	8.15	8.33
10	9.24	9.05
11	9.24	8.90
12	12.05	9.20
13	12.00	8.53
14	12.04	8.03
15	12.15	7.33
16	11.30	7.33
17	10.30	7.00
18	8.42	6.81
19	8.30	6.50
20	7.14	6.32
21	6.90	5.85

Antimicrobial activity test was carried out using gram-positive bacteria (*Staphylococcus aureus* ATCC 25923) and gram-negative bacteria (*Escherichia coli* ATCC 25922) for 21 days. The results of this screening show that this fermentate has inhibitory activity against *S. aureus* and *E. coli* (Table 1). The best activity against *S. aureus* was shown on day 15 with an inhibition zone diameter of 12.15

mm and against *E. coli* was shown on the 12 th day with a bland zone diameter of 9.20 mm.

Optimization of Fermentation Conditions and Antimicrobial Activity for Active Endophytic Fungi Isolates with Variations in pH and Medium Substrates in the Production of Fermentation Secondary Metabolites

The optimization of fermentation conditions for active endophytic fungal isolates was carried out based on variations in pH (4, 7, and 8). The test results show that pH 7 is the optimum pH in inhibiting bacterial growth *S. aureus* and *E. coli* with inhibition zone diameters of 13.15 mm and 11.29 mm, respectively (Figures 2 and 3).

The optimization of fermentation media conditions was carried out based on variations in carbon sources, using fructose and glucose. The test results show that fructose is a better carbon source than glucose (Table 2). The results of antimicrobial activity test using ethyl acetate extract from the fermentate of endophytic fungal show that the ethyl acetate extract from the fermentate of endophytic fungi resulting from optimization with a concentration of 60% has a weak inhibitory response to *S. aureus* and *E. coli* (Table 3).

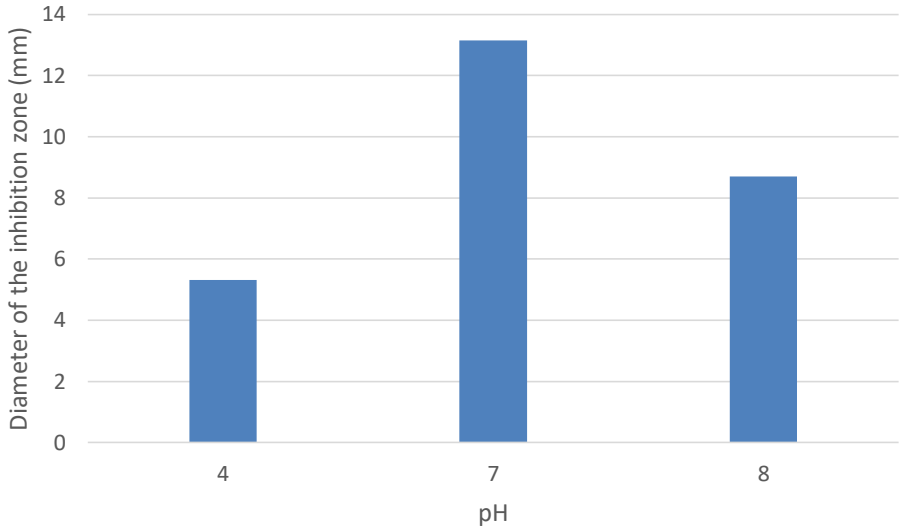

Figure 2. pH optimization profile against *S. aureus*

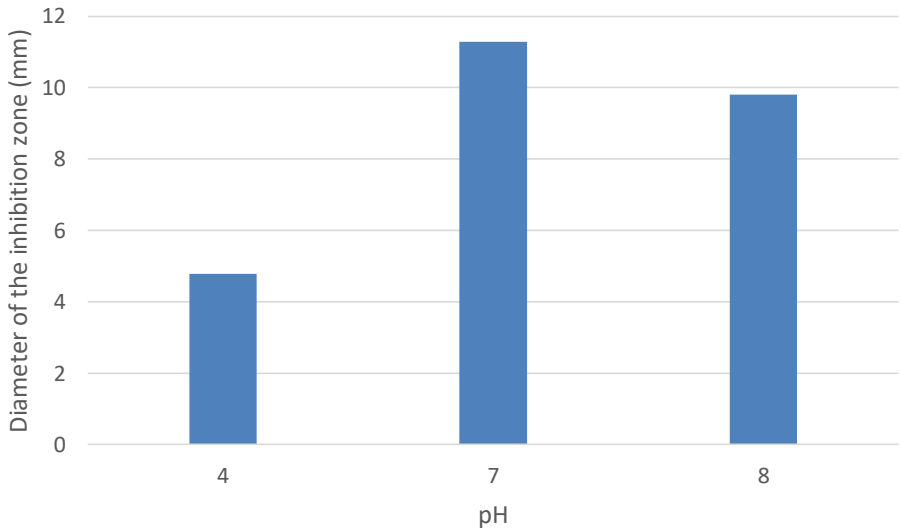

Figure 3. pH optimization profile against *E. coli*

Table 2. Source optimization profile against *S. aureus* and *E. coli*

Variations in carbon sources	Diameter of the inhibition zone (mm)					
	S. aureus			*E. coli*		
	pH 4	pH 7	pH 8	pH 4	pH 7	pH 8
Glucose	4.17	8.08	8.31	4.55	9.05	8.23
Fructose	5.15	11.88	10.9	4.33	10.15	9.00

Table 3. Fermentate activity test results from optimization of fermentation conditions based on differences in pH and carbon sources

Optimized fermentation media	Diameter of the inhibition zone (mm)	
	S. aureus	E. coli
Fructose + pH 7	13.50	11.35

Antioxidant Activity Test

Testing the antioxidant activity of the extract shows IC50 value ethyl acetate extract and vitamin C respectively is 24.29 and 14.99 µg/ml (Table 4). This value shows that the extract has very strong antioxidant activity.

Table 4. IC50 Value of Ethyl Acetate and Vitamin C Extract

Sample	IC50 (µg/ml) ± SD	Antioxidant Activity (Bahriul et al., 2014)
Ethyl Acetate Extract	24.29±013	Very strong
Vitamin C	14.99±013	Very strong

DISCUSSION

In previous research, 3 endophytic fungi were isolated from Kelakai. Based on identification results macroscopic and microscopic, the three isolates

were identified as the genus *Aspergillus* sp., *Paecilomyces sp.*, and *Arthrocristula* sp. The various endophytic fungi that can be isolated from kelakai show that every plant around us is a breeding ground for several endophytic fungi (Strobel, 2003). The number of types of endophytic functions that can be obtained from plants is greatly influenced by factors such as temperature, humidity, chemical variations, anatomical structure and age of the colonized host organs (García-Sánchez et al., 2020).

The isolate used was an isolate with the code Sp1Htm, identified as Aspergillus sp., which was the result of isolation in previous research (Mashar et al., 2023). This isolate code was chosen because it is the isolate that has the largest barrier zone compared to the other 3 isolates. The initial stage carried out was optimizing the production time of secondary metabolites. Optimization of production time aims to determine the right time to produce secondary metabolites optimally (Syarifuddin et al., 2021). Optimization was carried out by graphing the relationship between fermentation time and the diameter of the inhibition zone.

Production of secondary metabolites from endophytic fungi with the code Sp1Htm using PDY media. Fermentation was carried out using a closed system and the fungus used came from Potato Dextrose Agar (PDA) media which was 5 days old. PDY media was chosen because it can shorten the lag phase in fungal growth. PDY media has a similar composition to PDA media, the only difference is the consistency (Ginovart et al., 2011). The Sp1Htm fungus is expected to be able to adapt quickly to PDY media because it has previously adapted to PDA media. The purpose of shortening the lag phase is because a short lag phase will allow the fungus to enter the logarithmic phase more quickly, so it is hoped that the formation of secondary metabolites will occur more quickly in the stationary phase (Rolfe et al., 2012). Apart from that, the same content in PDY and PDA media will guarantee that the production results from the fermentation process are the same as those produced in PDA media. The fermentation process was carried out for 21 days, this was done to determine the growth of the mycelium. Apart from that, during these 21 days it is estimated that the stationary phase has been reached (Rumidatul et al., 2021). The achievement of the stationary phase is marked by a color change in the media, an increase in the viscosity of the media, and the volume of mycelia in the fermenter does not increase. PDY media is used in the fermentation process because it is more effective in increasing fungal cell biomass and secondary metabolites compared to fermentation on agar media. With liquid media, an agitation process can be carried out which plays a role in maintaining the homogeneity of nutrients in the media so that the process of absorbing nutrients by fungi becomes more optimal (Zhou et al., 2018). After reaching the 21st day, the mycelia and media were separated using a filtering technique (Singgih et al., 2017).

As a step to carry out initial identification of its ability as an antimicrobial, an antimicrobial activity test was carried out on the fermentate. The best activity against S. aureus was shown on day 15 with an inhibition zone diameter of 12.15 mm and against E. coli was shown on the 12th day with a

bland zone diameter of 9.20 mm. The diameter of the inhibition zone of a sample in the range of 7-11 mm is included in the weak activity category, while >16 mm is included in the strong activity category (Monks et al., 2002).

Next, optimization of fermentation conditions for active endophytic fungal isolates was carried out based on variations in pH (4, 7, and 8) and carbon sources, using fructose and glucose, in the production of fermentation secondary metabolites. This stage was carried out in accordance with the optimum time for growth of active isolates obtained previously. Optimization based on pH variations aims to determine the pH range isolates can grow and produce bioactive compounds optimally. pH plays an important role in enzymatic reactions and influences the growth of fermentation microorganisms and causes changes in the structure of bioactive compounds which can have an impact on biological activity, including antioxidants. At optimum pH, growth also stimulates the production of bioactive compounds (Bishop et al., 2022; Hur et al., 2014).

Different pH environments affect the type and quantity of metabolites produced by microbes. Neutral pH encourages the formation of organic acids by *Aspergillus* sp (Qayyum et al., 2019). In general, the optimal pH for the production of antimicrobial compounds ranges between 7 and 8. Changes in the pH of the growth medium for microorganisms have a bad impact on the production of antimicrobial compound metabolites.(Vijayakumar et al., 2012).

Next, optimization of fermentation media conditions was carried out based on variations in carbon sources, using fructose and glucose. Media is a source of nutrition for the growth of microorganisms, able to provide a source of carbon and other minerals needed for the growth of microorganisms (Andualem & Gessesse, 2013). Carbon sources are the main nutrients needed by fungi, playing a role in the life and metabolism of fungi. Different carbon sources were chosen in order to produce more organic acids (Qayyum et al., 2019).

Sucrose is the most popular carbon source in tissue culture. Sucrose has efficient absorption capabilities across the plasma membrane. Various carbon sources can be used in growth media. Media given monosaccharides such as glucose or sucrose can produce consistently high cultures of microorganisms with higher growth rates compared to fructose. The use of sucrose is a good carbon source for culturing microorganisms (Swamy et al., 2010). Based on these results, fungal isolates from the genus *Aspergillus* sp can grow optimally and show good antimicrobial activity in media conditions with pH 7 and fructose as a carbon source. The *Aspergillus* genus has nutritional potential in various environmental conditions, including host tissue. Gene expression of enzymes involved in metabolic processes allows fungi to be highly effective in upregulating the tricarboxylic acid cycle and increasingly able to metabolize other secondary carbon sources (Nji et al., 2023; Qayyum et al., 2019).

Environmental factors greatly influence the production of bioactive compounds in *Aspergillus* fungi, including pH and carbon sources. Suitable environmental conditions are needed to support the sporulation process and secretion of bioactive compounds (Calvo et al., 2002; Oliviero et al., 2022).

After the fermentation process ends, the optimized fermentation media is separated from the fungal myceliafractionatedwith ethyl acetate using a ratio of 1:1. The ethyl acetate fraction was separated and evaporated to obtain a thick extract. Ethyl acetate solvent is used because it has semipolar properties so it can dissolve polar and nonpolar bioactive compounds (Rumidatul et al., 2021).

The antimicrobial activity test using ethyl acetate extract from the fermentate of endophytic fungal samples was carried out on *S. aureus* and *E. coli* using the disk diffusion method. The extract concentration used is 60%. This concentration was chosen because previous research stated that the antimicrobial activity test results showed that Sp1Htm and Sp2HjK ethyl acetate extracts at concentrations of 15, 30 and 45% had a weak inhibitory response to *S. aureus* and *E. coli* that the concentration of the extract is increased.

Testing the antioxidant activity of the extract was compared with standard vitamin C using concentrations of 10, 15, 20, 25, and 30 µg/ml. The antioxidant capacity of the samples was identifiedusing the IC50 value parameter (Inhibitory Concentration 50%). IC50 value is a parameter that is widely used to measure the antioxidant activity of a sample. IC50 is the concentration of antioxidant compounds that can reduce DPPH activity by 50%. A lower IC50 value indicates high antioxidant activity (Akullo et al., 2023).

The IC50 value is obtained by making a linear equation between the test concentration and percent inhibition. If the IC50 value obtained is <50 µg/ml, it indicates that the antioxidant activity of the sample is in the very strong category (Bahriul et al., 2014). This shows that the kelakai endophytic fungus has great potential as an antioxidant that can be used for the development of raw materials for traditional medicine.

CONCLUSIONS AND RECOMMENDATIONS

The growth curve graph shows that exponential growth only begins on day 1 and reaches a maximum on day 17. Based on pH variations, it shows that pH 7 is the optimum pH, while based on variations in carbon sources, it shows that fructose is a better carbon source than glucose in inhibiting bacterial growth. Antimicrobial activity test using ethyl acetate extract showed antimicrobial activity against S. aureus and E. coli with the respective inhibition zone diameters being 13.50 mm and 11.35 mm. IC50 value Ethyl acetate extract is 24.29 µg/ml which shows that the extract has very strong antioxidant activity. However, other potential benefits remain to be explored in future research.

REFERENCES

Adeleke, B. S., & Babalola, O. O. (2021). Pharmacological potential of fungal endophytes associated with medicinal plants: A review. *Journal of Fungi*, *7*(2), 1–16. https://doi.org/10.3390/jof7020147

Aini, K., Elfita, E., Widjajanti, H., & Setiawan, A. (2022). Bioactivity Endophytic Fungi Isolated from the Leaf Stalk of Syzygium jambos L. Alston. *Tropical Journal of Natural Product Research*, *6*(11), 1765–1772. https://doi.org/10.26538/tjnpr/v6i11.4

Akullo, J. O., Kiage-Mokua, B. N., Nakimbugwe, D., Ng'ang'a, J., & Kinyuru, J. (2023). Phytochemical profile and antioxidant activity of various solvent extracts of two varieties of ginger and garlic. *Heliyon*, *9*(8), e18806. https://doi.org/10.1016/j.heliyon.2023.e18806

Andualem, B., & Gessesse, A. (2013). Production of microbial medium from defatted brebra (Milletia ferruginea) seed flour to substitute commercial peptone agar. *Asian Pacific Journal of Tropical Biomedicine*, *3*(10), 790–797. https://doi.org/10.1016/S2221-1691(13)60157-4

Bahriul, P., Rahman, N., & Diah, A. W. M. (2014). Uji Aktivitas Antioksidan Ekstrak Etanol Daun Salam (Syzyngium polyanthum) Dengan Metode DPPH. *Jurnal Akademika Kimia*, *3*(3), 368–374.

Bishop, P., Pitts, E. R., Budner, D., & Thompson-Witrick, K. A. (2022). Kombucha: Biochemical and microbiological impacts on the chemical and flavor profile. *Food Chemistry Advances*, *1*(March), 100025. https://doi.org/10.1016/j.focha.2022.100025

Calvo, A. M., Wilson, R. A., Bok, J. W., & Keller, N. P. (2002). Relationship between Secondary Metabolism and Fungal Development. *Microbiology and Molecular Biology Reviews*, *66*(3), 447–459. https://doi.org/10.1128/MMBR.66.3.447

Dewage, E., Sandun, N., Nam, K., Huang, X., & Ahn, D. U. (2022). Mechanisms , and Applications : A Review. *Antioxidants*, *11*(1025), 1–18.

Elfita, Oktiansyah, R., Mardiyanto, Widjajanti, H., & Setiawan, A. (2022). Antibacterial and antioxidant activity of endophytic fungi isolated from Peronema canescens leaves. *Biodiversitas*, *23*(9), 4783–4792. https://doi.org/10.13057/biodiv/d230946

García-Sánchez, A., Miranda-Díaz, A. G., & Cardona-Muñoz, E. G. (2020). The Role of Oxidative Stress in Physiopathology and Pharmacological Treatment with Pro- And Antioxidant Properties in Chronic Diseases. *Oxidative Medicine and Cellular Longevity*, *2020*.

https://doi.org/10.1155/2020/2082145

Ginovart, M., Prats, C., Portell, X., & Silbert, M. (2011). Exploring the lag phase and growth initiation of a yeast culture by means of an individual-based model. *Food Microbiology*, *28*(4), 810–817. https://doi.org/10.1016/j.fm.2010.05.004

Gouda, S., Das, G., Sen, S. K., Shin, H. S., & Patra, J. K. (2016). Endophytes: A treasure house of bioactive compounds of medicinal importance. *Frontiers in Microbiology*, *7*(SEP), 1–8. https://doi.org/10.3389/fmicb.2016.01538

Gurgel, R. S., de Melo Pereira, D. Í., Garcia, A. V. F., Fernandes de Souza, A. T., Mendes da Silva, T., de Andrade, C. P., Lima da Silva, W., Nunez, C. V., Fantin, C., de Lima Procópio, R. E., & Albuquerque, P. M. (2023). Antimicrobial and Antioxidant Activities of Endophytic Fungi Associated with Arrabidaea chica (Bignoniaceae). *Journal of Fungi*, *9*(8). https://doi.org/10.3390/jof9080864

Handayani, D., Rivai, H., Mulyana, R., Suharti, N., Rasyid, R., & Hertiani, T. (2018). Antimicrobial and cytotoxic activities of endophytic fungi isolated from mangrove plant Sonneratia alba Sm. *Journal of Applied Pharmaceutical Science*, *8*(2), 049–053. https://doi.org/10.7324/JAPS.2018.8207

Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I., & Kim, G. B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food Chemistry*, *160*, 346–356. https://doi.org/10.1016/j.foodchem.2014.03.112

Jia, M., Chen, L., Xin, H. L., Zheng, C. J., Rahman, K., Han, T., & Qin, L. P. (2016). A friendly relationship between endophytic fungi and medicinal plants: A systematic review. *Frontiers in Microbiology*, *7*(JUN), 1–14. https://doi.org/10.3389/fmicb.2016.00906

Mashar, H. M., Supriyono, T., Ismail, I., & Ysrafil, Y. (2023). Exploration of Endophytic Fungi in the Kelakai (Stenochlaena palustris) and Their Antibacterial Effect. *International Journal of Pharmaceutical Quality Assurance*, *14*(2). https://doi.org/10.25258/ijpqa.14.2.01

Michalak, M. (2022). Plant-Derived Antioxidants: Significance in Skin Health and the Ageing Process. *International Journal of Molecular Sciences*, *23*(2), 8–12. https://doi.org/10.3390/ijms23020585

Monks, N. R., Lerner, C., Henriques, A. T., Farias, F. M., Schapoval, E. E. S., Suyenaga, E. S., Da Rocha, A. B., Schwartsmann, G., & Mothes, B.

(2002). Anticancer, antichemotactic and antimicrobial activities of marine sponges collected off the coast of Santa Catarina, southern Brazil. *Journal of Experimental Marine Biology and Ecology*, *281*(1–2), 1–12. https://doi.org/10.1016/S0022-0981(02)00380-5

Mookherjee, A., Mitra, M., Kutty, N. N., Mitra, A., & Maiti, M. K. (2020). Characterization of endo-metabolome exhibiting antimicrobial and antioxidant activities from endophytic fungus Cercospora sp. PM018. *South African Journal of Botany*, *134*, 264–272. https://doi.org/10.1016/j.sajb.2020.01.040

Nji, Q. N., Babalola, O. O., & Mwanza, M. (2023). Soil Aspergillus Species, Pathogenicity and Control Perspectives. *Journal of Fungi*, *9*(7), 1–16. https://doi.org/10.3390/jof9070766

Oliviero, M., Langellotti, A. L., Russo, G. L., Baselice, M., Donadio, A., Ritieni, A., Graziani, G., & Masi, P. (2022). Use of Different Organic Carbon Sources in Cynara cardunculus Cells: Effects on Biomass Productivity and Secondary Metabolites. *Plants*, *11*(5). https://doi.org/10.3390/plants11050701

Pandy, R., Kumar, S. S., Suresh, P., Annaraj, J., Pandi, M., Vellasamy, S., & Sagadevan, S. (2023). Screening and characterization of fungal taxol-producing endophytic fungi for evaluation of antimicrobial and anticancer activities. *Open Chemistry*, *21*(1). https://doi.org/10.1515/chem-2022-0344

Praptiwi, Raunsai, M., Wulansari, D., Fathoni, A., & Agusta, A. (2018). Antibacterial and antioxidant activities of endophytic fungi extracts of medicinal plants from Central Sulawesi. *Journal of Applied Pharmaceutical Science*, *8*(8), 069–074. https://doi.org/10.7324/JAPS.2018.8811

Preuttiporn, S., & Sita, P. (2019). Antimicrobial and antioxidant activities of endophytic fungi extracts isolated from Carissa carandas. *African Journal of Microbiology Research*, *13*(27), 464–473. https://doi.org/10.5897/ajmr2019.9164

Qayyum, S., Meng, K., Pervez, S., Nawaz, F., & Peng, C. (2019). Optimization of pH, temperature and carbon source for bioleaching of heavy metals by Aspergillus flavus isolated from contaminated soil. *Main Group Metal Chemistry*, *42*(1), 1–7. https://doi.org/10.1515/mgmc-2018-0038

Rammali, S., Hilali, L., Dari, K., Bencharki, B., Rahim, A., Timinouni, M., Gaboune, F., El Aalaoui, M., & khattabi, A. (2022). Antimicrobial and antioxidant activities of Streptomyces species from soils of three different cold sites in the Fez-Meknes region Morocco. *Scientific Reports*,

12(1), 1–22. https://doi.org/10.1038/s41598-022-21644-z

Rolfe, M. D., Rice, C. J., Lucchini, S., Pin, C., Thompson, A., Cameron, A. D. S., Alston, M., Stringer, M. F., Betts, R. P., Baranyi, J., Peck, M. W., & Hinton, J. C. D. (2012). Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *Journal of Bacteriology*, *194*(3), 686–701. https://doi.org/10.1128/JB.06112-11

Rumidatul, A., Rahmawati, N., & Sunarya, S. (2021). Production of secondary metabolites and its antibacterial and antioxidant activity during the growth period of endophytic fungi isolated from gall rust sengon plants. *Pharmacognosy Journal*, *13*(2), 325–331. https://doi.org/10.5530/pj.2021.13.42

Saleh, A. M., El-Refai, H. A., Hashem, A. M., El-Menoufy, H. A., Mansour, N. M., & El-Beih, A. A. (2019). Optimization Studies and Chemical Investigations of Aspergillus terreus-18 Showing Antioxidant Activity. *Egyptian Journal of Chemistry*, *62*(2), 215–230. https://doi.org/10.21608/ejchem.2018.4921.1438

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. El, Polito, L., Iriti, M., Martins, N., Martorell, M., Docea, A. O., … Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Frontiers in Physiology*, *11*(July), 1–21. https://doi.org/10.3389/fphys.2020.00694

Singgih, M., Julianti, E., & Radzali, M. D. (2017). Isolation and Antibacterial Activity of Soil-Derived Fungi From Taman Botani Negara, Shah Alam, Malaysia. *Acta Pharmaceutica Indonesia*, *42*(1), 18–24. https://doi.org/10.5614/api.v42i1.4511

Strobel, G. A. (2003). Endophytes as sources of bioactive products. *Microbes and Infection*, *5*(6), 535–544. https://doi.org/10.1016/S1286-4579(03)00073-X

Swamy, M. K., Sudipta, K. M., Balasubramanya, S., & Anuradha, M. (2010). Effect of Dfferent Carbon Sources on in vitro Morphogenetic Response of Patchouli (Pogostemon cablin Benth). *Journal of Phytology*, *2*(8), 11–17. www.journal-phytology.com

Syarifuddin, A., Sulistyani, N., & Wijayatri, R. (2021). *The Optimization of Antibacterial Production Time of Actinomycetes (J4 Isolate) Against Staphylococcus Aureus and Escherichia Coli*. https://doi.org/10.4108/eai.18-11-2020.2311633

Utami, L. A., Wahyuni, W. T., Mubarik, N. R., & Astuti, R. I. (2023). Endophytic bacteria of clove (Syzygium aromaticum L.) leaves produce metabolites with antioxidant and anti-aging properties. *Journal of Applied Pharmaceutical Science*, *13*(7), 241–250. https://doi.org/10.7324/JAPS.2023.93258

Vijayakumar, R., Panneerselvam, K., Muthukumar, C., Thajuddin, N., Panneerselvam, A., & Saravanamuthu, R. (2012). Optimization of Antimicrobial Production by a Marine Actinomycete Streptomyces afghaniensis VPTS3-1 Isolated from Palk Strait, East Coast of India. *Indian Journal of Microbiology*, *52*(2), 230–239. https://doi.org/10.1007/s12088-011-0138-x

Widjajanti, H., Nurnawati, E., Muharni, & Zahwa, E. D. (2022). Optimization of Antibacterial Production of Endophytic Fungi with Various Sources of C, N, and pH using The Response Surface Methodology. *Science and Technology Indonesia*, *7*(2), 149–157. https://doi.org/10.26554/sti.2022.7.2.149-157

Zhao, S., Wu, X., Duan, X., Zhou, C., Zhao, Z., Chen, H., Tang, Z., Wan, Y., Xiao, Y., & Chen, H. (2021). Optimal extraction, purification and antioxidant activity of total flavonoids from endophytic fungi of Conyza blinii H. Lév. *PeerJ*, *9*. https://doi.org/10.7717/peerj.11223

Zhou, Y., Han, L. R., He, H. W., Sang, B., Yu, D. L., Feng, J. T., & Zhang, X. (2018). Effects of agitation, aeration and temperature on production of a novel glycoprotein gp-1 by streptomyces kanasenisi zx01 and scale-up based on volumetric oxygen transfer coefficient. *Molecules*, *23*(1), 1–14. https://doi.org/10.3390/molecules23010125