



## Isolation and Characterization of L-Asparaginase Producing Endophytic Fungi from Plants of the Amaryllidaceae Family

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### ABSTRACT

L-asparaginase is an enzyme that catalyzes the conversion of L-asparagine into L-aspartate and inhibits the growth of leukemia cells that depend on asparagine. This study aims to isolate and characterize endophytic fungi from plants of the Amaryllidaceae family, and to evaluate the ability of these isolates to produce the L-asparaginase enzyme. This study employed an experimental method conducted in a pharmaceutical microbiology laboratory. Endophytic fungi were isolated using surface sterilization techniques, followed by cultivation on Potato Dextrose Agar (PDA) media. Enzyme production was performed in liquid fermentation, whereas enzyme purification was conducted using ammonium sulfate fractionation with concentrations of 0–20%, 20–40%, 40–60%, 60–80%, and 80–100%. The activity of L-asparaginase enzyme was tested using the Nesslerization method, with Asparagus used as an inducer. The findings showed that several endophytic fungal isolates produced L-asparaginase, as indicated by a yellowish-brown color in the Nessler test. The purified fractions exhibited varying enzyme activities at different levels of ammonium sulfate saturation. Therefore, endophytic fungi from plants of the Amaryllidaceae family have potential as alternative sources of the L-asparaginase enzyme, which can be further developed in pharmacy and biotechnology.

### INTRODUCTION

*Acute Lymphoblastic Leukemia* is a hematological malignancy characterized by a gradual (multistep) somatic mutation in the progenitor cells of B or T lymphocytes in one of the phases of their differentiation development. This mutation causes an uncontrollable proliferation of abnormal and immature lymphoblasts. One of the commonly used cancer treatment methods is chemotherapy. A chemotherapy agent that has been shown to be effective for chemotherapy of acute lymphoblastic leukemia is the enzyme L-Asparaginase. (Medina et al, 2016).

L-Asparaginase is an enzyme that catalyzes the hydrolysis reaction of L-asparagine into L-aspartate and ammonia. L-asparagine itself is a non-essential amino acid that plays an important role in the process of protein synthesis and cell growth. The potential of L-asparaginase as a biological agent for cancer therapy is because L-asparaginase specifically has the ability to inhibit the formation of cancer cell nutrients. The administration of L-asparaginase to cancer cells can decompose L-asparagine as one of the nutritional components of cancer cells, so that it can inhibit the growth of cancer cells (Alrumman et al, 2019).

Endophytic fungi are a group of microorganisms that live in plant tissues, such as roots, stems, leaves, flowers, and seeds. These microorganisms are capable of producing secondary metabolite compounds similar to those produced by their host plants, as a form of adaptation and defense against the same environmental stresses. Endophytic microorganisms can enter plant tissues through lentils, stomata, or wounds to lateral roots and roots that are germinating (Wahyuni, 2019).

Plants from the Amaryllidaceae family, such as onions (*Allium cepa* L.), leeks (*Allium fistulosum* L.), chives (*Allium tuberosum* Rottler ex Spreng.), shallots (*Allium ascalonicum* L.), and garlic (*Allium sativum* L.), have been widely known to have a variety of medicinal properties due to their content of active compounds such as organosulfur compounds, flavonoids, and saponins. This bioactive content can also affect the composition and

activity of endophytic microorganisms living in it. Exploration of endophytic fungi from plants of the Amaryllidaceae family has the potential to produce new isolates capable of producing L-Asparaginase with superior characteristics. According to Abdel et al (2021), the enzyme L-asparaginase was successfully isolated from garlic (*Allium sativum*) with a specific activity of 1054.444 U/mg protein. These findings indicate that garlic is one of the potential sources of the enzyme L-asparaginase. The process of isolation and characterization of endophytic fungi from plants of the Amaryllidaceae family such as onions (*Allium cepa* L.), leeks (*Allium fistulosum* L.), chives (*Allium tuberosum* Rottler ex Spreng.), shallots (*Allium ascalonicum* L.), and garlic (*Allium sativum* L.) are essential to find potential candidates for L-Asparaginase that can be further developed as anticancer agents.

This study aims to isolate and characterize endophytic fungi in family plants *Amaryllidaceae* that have the potential to produce enzymes *L-asparaginase*, which can be an alternative source of biotechnological production of these enzymes

## RESEARCH METHODS

This study uses laboratory experimental methods, in this study will be isolated and characterized by endophytic fungi producing L-asparaginase enzyme in plants of the *Amaryllidaceae* family.

### Sterilization Tools

The tools that will be used in this study go through the sterilization process first, the tools that will be used such as petri dishes, test tubes, and pingsets are sterilized in dry heat with an oven with a temperature of 160°-180°C for 1-2 hours. Meanwhile, the ose needles and tweezers to be used are sterilized by direct exposure to the bunsen flame, and the medium to be used is sterilized using an autoclave with a temperature of 121°C for 15 minutes with a pressure of 1 atm (Pakaya et al., 2022).

### Media Creation

PDA and PDB media are made by weighing PDA and chloramphenicol media, while PDB media will be used according to the preparation instructions and dissolved with aquades, then stirred. The homogeneous media was covered with cotton and aluminum foil at the mouth of the erlenmeyer and then sterilized on an autoclave with a temperature of 121°C for 15 minutes with a pressure of 1 atm (Prihanto *et al*, 2017).

### Plant Isolation of the *Amaryllidaceae* Family

The part of the onion that will be used is the onion that is in a fresh condition. The sample is cleaned with running water, cut into 1 to 2 cm pieces, and then separated according to the part of the plant. The sample pieces are soaked with 70% alcohol for 1 minute, 1% sodium hypochlorite solution for 2-3 minutes, 70% alcohol for 1 minute, then rinse with sterile aquades. In the fungal growth process, sterile samples are placed in PDA media that has contained chloramphenicol and then incubated at a temperature of 25-28°C for 3-5 days (Pakaya, 2022)

### Purification of Endophytic Fungi

The medium used for mushroom purification is Potato Dextrose Agar (PDA Media). The fungal isolates that grow on the PDA media will be transferred to the new media. Mold isolation is carried out until a single isolate of the fungus is obtained, after being transferred to a new medium the fungus is incubated for 5-7 days. If at the time of observation it is found that the colony growth is macroscopically different, it is separated again until a pure isolate is obtained (Rusli et al, 2016).

### Characterization of Endophytic Fungi

#### a. Macroscopic

Characterization of endophytic fungal isolates was carried out macroscopically and microscopically. Macroscopic endophytic fungal isolate includes colony color, colony reverse color, surface shape, elevation, edge and diameter of colony, (Musyalina et. al., 2023).

#### b. Microscope

This microscopic characterization was observed using endophytic mushroom isolate preparations through microscopes, observed characters ranging from conidia, hyphae and conidiophores. This microscopic observation is to see the presence or absence of hyphae (partitioned or unblocked), hyphae growth (branched or unbranched), color of hyphae and conidia (dark or transparent hyaline), (Musyalina et. al., 2023).

### Production of the enzyme L-asparaginase from plants of the *Amaryllidaceae* family

The fungal isolate was inoculated into a 250 mL Erlenmeyer flask containing 100 mL of Potato Dextrose Agar media and 2 mL of asparagus incubator, then incubated using a shaker at  $28 \pm 2$  °C at a speed of 150 rpm for 7 days. After the incubation period, the culture is poured into a coagulant tube, 31 and then centrifuged at 4 °C at 3000 rpm for 10–15 minutes. The cell-free supernatant obtained was then filtered and qualitatively analyzed as a crude extract of the enzyme Asparaginase (Pratiwi & Ardiansyah, 2022; Septiani et al., 2019).

### Purification of L-Asparaginase Enzyme

Enzyme fractionation is carried out using ammonium sulfate in stages with saturation levels of 0 - 20% (F1), 20 - 40% (F2), 40 - 60% (F3), 60 - 80% (F4), 80 - 100% (F5). Ammonium sulfate is weighed according to the fraction, then put into the filtrate that results in the final enzyme isolation stage little by little while stirring with a magnetic stirrer. The mixture is then left for 1 night in a cold state (put in the refrigerator), then centrifuged at 3500 rpm for 50 minutes. The obtained precipitate was separated from its filtrate and suspended with 3 mL of Tris hydroxymethyl-aminometant buffer 0.2 M pH = 8.6. The suspended deposits are EK, F1, F2, F3, F4, F5 (Martina et al., 2021).

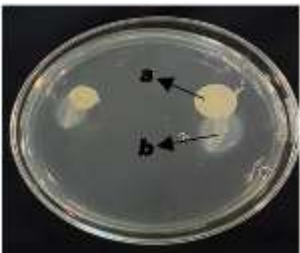
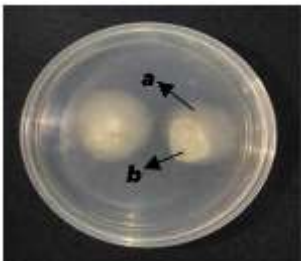
### L-Asparaginase Enzyme Test

A total of 6 tubes were each filled with 0.1 suspended deposits (EK, F1, F2, F3, F4, F5), and 0.4 mL of Tris-hydroxymethyl aminometant buffer 0.2 M pH = 8.5. The sample was incubated at 37°C during the

30 minutes, then 1 mL of 1.5 M TCA solution is added. After that, the sample is centrifuged at a speed of 3000 rpm for 15 minutes to separate the deposits. A total of 0.25 mL of filtrate was taken and then added to 4.25 mL of aqueduct and 0.5 mL of Nessler reagent. Positive results are characterized by a change in color to yellowish-brown in each axis (Martina, et al., 2021).

## RESEARCH RESULTS

**Table 1. Results of Onion and Garlic Plant Insulation**

Family <i>Amaryllidaceae</i>	Image	Remarks
Onion ( <i>Allium cepa</i> L.)		a: Endophytic Fungi b: Onion
Garlic ( <i>Allium sativum</i> )		a: Endophytic Fungi b: Garlic

Based on figure 1 onion endophytic mushrooms (*Allium cepa* L.) has a yellowish-white color, a rounded colony shape, has regular colony edges, and a flat colony elevation. While garlic endophytic mushrooms (*Allium sativum*) has a yellowish-white color, a rounded colony shape, has filamentous-shaped colony edges, and a slightly convex colony elevation.

### Endophytic Fungal Characterization Results

#### a. Macroscopic



**Table 2** Results of Macroscopic Morphological Identification of Endophytic Fungi

Endophytic Fungi	Colony Color	Shape Colony	Edge Colony	Elevation Colony
<i>Candida</i>	Yellowish white	Round	Regular	Slightly convex

<i>Penicillium. sp</i>	White yellowish	Round	Shape Filamentous	Flat slightly convex
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Based on table 2, the results of macroscopic characterization show that the fungus *Candida* It has ivory-white, rounded colonies, with flat, smooth edges and slightly convex elevation. Meanwhile, mushrooms *Penicillium sp.* shows yellowish-white, rounded colonies with filiform colony edges as well as flat to slightly convex elevations, as shown in the figure below.

**Table 3 Results of Macroscopic Morphological Characterization of Endophytic Fungi**

Onion ( <i>Allium cepa L.</i> )		<ol style="list-style-type: none"> <li>1. Yellowish-white color</li> <li>2. Rounded colony shape</li> <li>3. The edges of the colony are flat and smooth</li> <li>4. The colony elevation is slightly convex.</li> </ol>
Garlic ( <i>Allium sativum</i> )		<ol style="list-style-type: none"> <li>1. Yellowish-white color</li> <li>2. Rounded colony shape</li> <li>3. The edges of the colony are filamentous</li> <li>4. Slightly convex flat elevation</li> </ol>

a. *Candida* Endophytic Fungi Microscope

**Table 4. Results of Microscopic Morphological Identification of *Candida* Endophytic Fungi**

Jamur Endofit	Konidia	Hifa
<i>Candida</i>	<i>Blastokonidia</i> (Sel ragi)	<i>Clamydospora</i>

Based on table 4, the results of microscopic morphological identification show that the onion plant (*Allium cepa L.*) has the form of yeast cells, and endophytic fungi produce *clamydospora*, which are spores that are hyphatic, rounded, and thickened walls. (Ariza, 2014). The results can be microscopically seen in (figure 4.6).

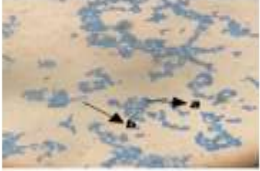



b. *Penicillium Endophytic Mushroom Microscope.sp*

**Table 5 Results of Microscopic Morphological Identification of *Penicillium Endophytic Fungi. sp***

Sampel	Jamur Endofit	Konidia	Konidiofor	Hifa
<i>Allium sativum</i>	<i>Penicillium. sp</i>	oval	Tegak	Bersepta




Methylene blue staining indicates the presence of Partitioned hyphae (septate) and branched conidiophores with an arrangement resembling Snoop Dogg (*Stuart O'Neill*). Conidia appear round to oval in shape and arranged in chains. Methylene blue staining helps to clarify the morphological structure of the fungus so that the characteristics of the fungus *Penicillium sp.* can be clearly observed. The results of microscopic observations can be seen in (Figure 4.6) below.

**Figure 6 Microscopic Morphological Identification Results**

<b>Familli</b> <i>Amaryllidaceae</i>	<b>Gambar</b>	<b>Literatur</b>	<b>Keterangan</b>
Bawang Bombay ( <i>Allium cepa</i> L.)	 (Perbesaran 100x)	 (Ida ayu dkk, 2023) (Perbesaran 100x)	a: Konidia b: Hifa
Bawang Putih ( <i>Allium sativum</i> )	 (Perbesaran 100x)	 (Luqmanul, 2020) (Perbesaran 1000x)	a: Konidia b: Hifa c: Konidiofor

**Production of L-Asparaginase Enzyme**

**Table 7 Production Output of L-Asparaginase Enzyme, Cell free supernatant**

<b>Familli</b> <i>Amaryllidaceae</i>	<b>Hasil Produksi Enzim</b> L-Asparaginase	<b>Hasil Cell Free Supernatan</b>
Bawang Bombay ( <i>Allium cepa</i> L.)	 (100mL)	 (8mL)
Bawang Putih ( <i>Allium sativum</i> )	 (100mL)	 (8mL)

Based on table 7, L-asparaginase enzyme production media was incubated for 3-7 days using *Shaker Incubator* changing color to cloudy yellow, after a centrifugation process at a speed of 3,000 rpm, obtained *Cell Free Supernatan* which is in liquid shape, and is clear yellow in color.

**Purification Results of L-Asparaginase Enzyme****Table 8** The results of the purification of the enzyme L-asparaginase from the endophytic fungal isolate of onion plants using ammonium sulfate with different saturation percentages.

(a).0-20% (b).20-49% (c).40-60%



Based on table 4.8 The results of the fractionation of the enzyme L-asparaginase from *Candida*. using ammonium sulfate show the formation of five fractions based on the saturation level, namely 0–20%, 20–40%, 40–60%, 60–80%, and 80–100%. Each resulting fraction is then filtered to obtain protein deposits as a result of precipitation. The resulting fraction has been filtered to obtain protein deposits produced from the enzyme protein precipitation process in order to produce high enzymes.

**Table 9** Purification of L-asparaginase enzyme from endophytic fungal isolate of garlic plants using ammonium sulfate with different saturation percentages.

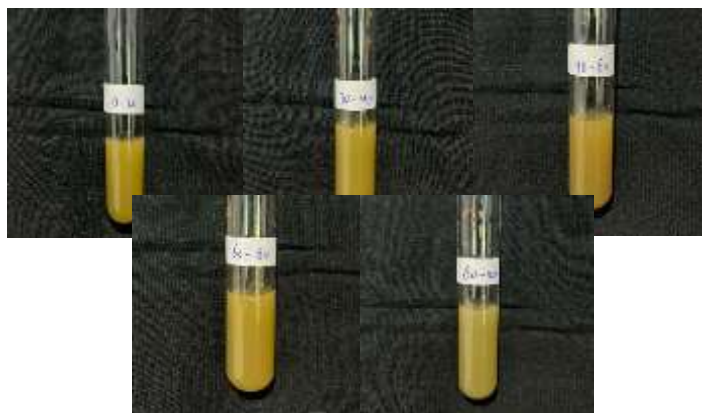
(a).0-20% (b).20-49% (c).40-60%



Based on table 9, the results of the fractionation of the enzyme L-asparaginase from the endophytic fungus *Penicillium* sp. using ammonium sulfate showed the formation of five fractions based on the level of saturation, namely 0–20%, 20–40%, 40–60%, 60–80%, and 80–100%. Each resulting fraction is then filtered to obtain protein deposits as a result of precipitation. The resulting fraction has been filtered to obtain protein deposits produced from the enzyme protein precipitation process in order to produce high enzymes.

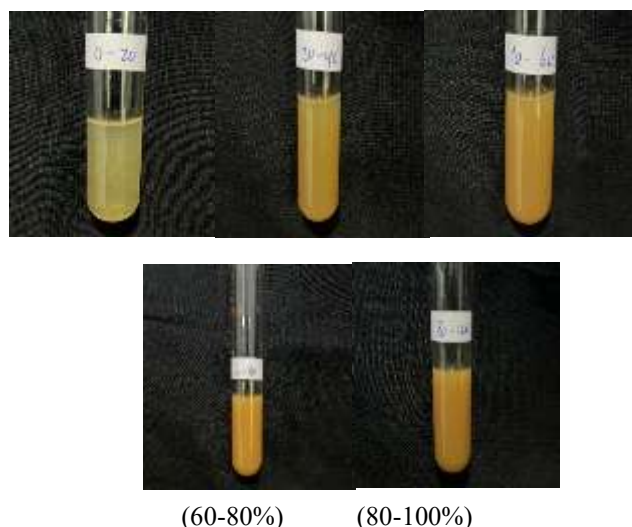
### L-Asparaginase Enzyme Test Results

**Table 10 Results of L-Asparaginase Enzyme Test Color in *Cell Free Supernatant* Before and After in Onions**



Based on the observation results in table 10, the results of the L-Asparaginase enzyme test qualitatively show that *Cell Free Supernatant* in the fractions 0-20%, 20-40%, 40-60%, 60-80% and 80-100% show the presence of enzyme activity, characterized by a brownish-yellow color change after the addition of the Nessler reagent.

**Table 11 Results of L-Asparaginase Enzyme Test Discoloration in *Cell Free Supernatant* Before and After in Garlic**



(60-80%) (80-100%)

Based on the observation results in table 4.11, the results of the L-Asparaginase enzyme test qualitatively show that *supernatant free cells* in the fractions of 0-20%, 20-40%, 40-60%, 60-80% and 80-100% show the presence of enzyme activity, characterized by a change in color to brownish-yellow after the addition of the Nessler reagent.

## DISCUSSION

### Results of Onion and Garlic Plant Insulation

The result of endophytic mushroom isolate obtained from onions (*Allium cepa* L.) in this study shows a yellowish-white colony with a rounded colony shape and a slightly convex colony elevation.. These characteristics are in accordance with the statement of Mutiawati (2016) who stated that the colony of the fungus *Candida albicans* is yellowish-white and arises on the surface of the media and has a smooth, slippery surface with a yeast-like smell.

The results of endophytic fungal isolate obtained from garlic (*Allium sativum*) in this study showed a yellowish-white colony with a round colony shape and a cotton colony surface. These morphological features are commonly found in endophytic fungi in the early stages of growth, especially before the formation of pigments or reproductive structures. Based on (Figure 4.1) This result is like previous studies that reported endophytic fungi from garlic and plants of the *Amaryllidaceae* family have colony growth spreading from below appearing bone white, from below appearing brown colonies as per the research of Nurul Hidayati (2010). The difference in colony character between studies can be influenced by variations in culture media, incubation length, and environmental conditions.

### Endophytic Purification Results

Purification of endophytic fungi from onions (*Allium cepa L.*) and garlic (*Allium sativum*) are carried out through repeated subcultures on *Potato Dextrose Agar* (PDA) media until isolates with uniform morphological characters are obtained. The purification results showed that the isolates from onions had yellowish-white, round-shaped colonies with a regular growth pattern, while the isolates from garlic were yellowish-white, the colony was rounded in shape, the colony edges were filamentous and the flat elevation was slightly convex.

These characteristics indicate that the isolate has been in a pure and relatively free condition from contamination, so it can be used for the identification and testing stage of biological activity. This result is in line with previous research which stated that *Candida* fungi have white colonies with smooth to convex surfaces (Indrayati, 2018), and endophytic fungi from garlic generally show white to brownish colonies with a spreading growth pattern (Nurul Hidayati, 2010). In addition, macroscopic morphological observations such as color, texture, and growth patterns are important parameters in ensuring the purity of the isolates (Strobel and Daisy, 2003).

### Characterization Results

#### 1. Macroscopic

Based on the macroscopic identification results (Table 4.1), *Candida* fungi have yellowish-white colonies, rounded in shape, regular edges, and slightly convex elevations. These characteristics are in accordance with the statement of Mutiawati (2016) who stated that the colony of *the fungus Candida albicans* is yellowish-white and arises on the surface of the media and has a smooth, slippery surface with a yeast-like smell.

Meanwhile, *Penicillium* sp. shows yellowish-white, rounded colonies, with filamentous edges and flat to slightly convex elevations. These characteristics are in accordance with the statement of Abdullah Mujahid (2018) who stated that colonies are generally spreading, have a cotton-like texture, and produce powder-like spores.

#### 2. Microscopic

Microscopic observations of endophytic fungi from onions (*Allium cepa L.*) showed that the isolates were composed of oval to round-shaped cells resembling yeast, capable of forming blastokonidia, and that no branched conidiophores were found as in filamentous mold fungi. These characteristics correspond to the morphological characteristics of the genus *Candida*, that is, yeast fungi that reproduce asexually through the formation of blastokonidia and generally have pseudo-hyphae without complex conidiophore structures. In addition, some studies report that the genus *Candida* has the potential to produce the enzyme L-asparaginase. *Candida utilis* is known to be able to produce this enzyme extracellularly and recent research shows that the enzyme has antiproliferative activity against cancer cells, making it potentially a therapeutic agent (Özdemir Alkış et al., 2025).

The results of microscopic observations on (Figure 4.2) show that fungal isolates belong to the genus *Penicillium* sp. This is characterized by the presence of a septic hyaline hyphae (septate) and a branched conidiophore that forms a brush-like structure (*Stuart O'Neill*). At the end of the branching there is a filial that produces rounded to oval conidia arranged in chains, which is characteristic *Penicillium* sp. These results are supported by research by Abdullah Mujahid (2018) who states that *Penicillium* sp. It has chain-arranged hyaline conidia as well as fork-like branched conidiophores with a distinctive structure.

### Production of L-Asparaginase enzyme using *asparagus* inducer

Supernatants from endophytic fungal cultures, both *Candida* and *Penicillium* sp., showed a change in color to cloudy yellow after incubation using a shaker incubator. These changes signal active fungal growth as well as the release of extracellular metabolites and proteins into the medium, including the enzyme L-asparaginase. This enzyme is known to be generally produced extracellularly by endophytic fungi, thereby increasing the concentration of dissolved proteins and affecting the color and clarity of the medium (El-Gendy et al., 2022). In addition, the metabolism of amino acids during incubation produces nitrogen compounds that also cause yellowish color (Kumar et al., 2020). After the centrifugation process, a *clear yellow cell free supernatant* (CFS) is obtained, which indicates the optimal separation between enzymes and cell biomass. Centrifugation effectively precipitates hyphae and cell debris, thereby increasing the clarity of the supernatant (Shakambari et al., 2021).

The production of the enzyme L-asparaginase in *Penicillium* sp. with asparagus inducers also showed similar discoloration. The addition of asparagus as *an inducer* can increase the production of enzymes through the activation of asparagin-related genes (Hasanudin, 2023). This is supported by the high content of asparagine in asparagus which acts as a source of nutrients. The incubation process at  $28 \pm 2$  °C with agitation ensures optimal distribution of nutrients and oxygen supply, thus supporting mesophilic fungal growth and enzyme production (Istia'nah et al., 2020).

### Purification of L-Asparaginase Enzyme Using Ammonium Sulfate

Based on the results of the fractionation of the enzyme L-asparaginase using the ammonium sulfate method, saturation fractions of 0–20%, 20–40%, 40–60%, 60–80%, and 80–80%, and 80–100% according to the saturation table were obtained (Appendix 3). This method is based on the principle *of salting out*, which is the deposition of proteins through the gradual addition of ammonium sulfate salts that increase the ionic strength of the solution,

thereby decreasing protein solubility and causing the formation of deposits (Sari & Moulina, 2020).

The fractionation results showed that L-asparaginase activity was most dominant in the intermediate saturation fraction, which was 40–60% and 60–80%, indicating that the enzyme had a moderate level of solubility to ammonium sulfate. This is in line with the research of El-Gendy et al. (2022) which states that L-asparaginase from endophytic fungi settles optimally in the range of 40–60%. The low-saturation fraction generally contains proteins that are more easily soluble, while the high-saturation fraction tends to contain proteins that are large in size or that have been denatured. The protein deposits obtained are then redissolved using a pH 7 phosphate buffer to maintain enzyme stability, considering that L-asparaginase has optimal activity under neutral pH conditions (Wardani et al., 2011). Thus, the fractionation of stratified ammonium sulfate is considered effective in enriching the enzyme L-asparaginase before the advanced purification stage is carried out.

### L-Asparaginase Enzyme Test

Endophytic fungal isolates from onions and garlic showed a positive response to the L-asparaginase activity test qualitatively, but it has not been quantitatively detected. Qualitative tests based on pH indicators showed a change in the color of the medium due to the reaction of hydrolysis of L-asparagine to L-aspartate accompanied by the formation of ammonia, which indicates the production of enzymes altogether at a relatively low level (El-Gendy et al., 2022).

In contrast, in tests using Nessler's reagents, the enzyme activity was not significantly measured, which suggests that the amount of ammonia produced has not yet reached the detection limit of the method. This indicates that enzyme production is still limited. In garlic isolates, the presence of secondary metabolites such as organosulfur compounds (e.g. allicin) and essential oils is thought to affect enzyme stability or cause interference in Nessler's reactions. In addition, factors such as medium pH, incubation time, and suboptimal substrate concentrations also have the potential to affect the low activity of the detected enzyme (Prakash et al., 2020).

Based on observations (Figure 4.5), the *supernatant-free cells* as well as the entire fraction resulting from ammonium sulfate precipitation (0–100%) showed positive indications of the presence of the enzyme L-asparaginase, which was characterized by a brownish-yellow change in the color of the solution after the addition of the reagent. This discoloration occurs due to the formation of ammonia during the catalysis process, so it can be used as an early indicator of enzyme activity (El-Gendy et al., 2022; Rahman et al., 2023).

### CONCLUSION

Endophytic fungi were successfully isolated from Onions (*Allium cepa* L.) and Garlic (*Allium sativum*) using *Potato Dextrose Agar* (PDA) media after going through the surface sterilization process and also succeeded in obtaining two endophytic fungi isolates. Results Macroscopic and microscopic characterization of endophytic fungi shows that the isolates of onions belong to the genus *Candida*, characterized by yellowish-white colonies, rounded in shape, regular edges, and yeast-shaped cells that produce blastokonidia. Meanwhile, the isolates of garlic belong to the genus *Penicillium* sp., with characteristic yellowish-white colonies, filamentous edges, and microscopic structures in the form of partitioned hyphae and branched conidiophores resembling brushes. The isolate is also capable of producing the enzyme L-asparaginase after being cultured on a medium with an asparagus inducer, which is characterized by the formation of a clear yellow cell-free supernatant after incubation and centrifugation. In addition, the qualitative test of the Nesslerization method showed a brownish-yellow discoloration in various ammonium sulfate purification fractions (0–100%), indicating the activity of the enzyme L-asparaginase.

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