



Isolation and Phytochemical Testing of Endophytic Fungi from *Imperata cylindrica* (L.) Beauv plants.

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ABSTRACT

High drug prices and increasing antibiotic resistance have prompted exploration of natural resources as a source of new antibiotics. The *Imperata cylindrica* (L.) Beauv. plant is one of the plants used in traditional medicine. Secondary metabolites as bioactive compounds in plants can be obtained through endophytic fungi without having to extract them from plants. Secondary metabolites produced by endophytic fungi can function as raw materials for medicines. This research aims to isolate endophytic fungi and test phytochemicals from *Imperata* plants to determine the type of secondary metabolites produced. The methods used in this research include isolation and purification of endophytic fungi, identification of endophytic fungi, production of secondary metabolites of endophytic fungi, and screening of phytochemicals resulting from endophytic fungi fermentation. The isolation results showed that there were 7 isolates of endophytic fungi, consisting of 1 isolate from the flower, 3 isolates from the stem, and 3 isolates from the roots. Identification of endophytic fungus using macroscopic and microscopic observations including the genera *Fusarium* sp., *Mucor* sp., *Penicillium* sp., *Trichoderma* sp., *Aspergillus* sp., *Phytophthora* sp. The results of the phytochemical test for endophytic fungi showed that the secondary metabolites produced were flavonoid and saponin compounds. Based on this research, it can be concluded that the endophytic fungi of the *Imperata cylindrica* (L.) Beauv. plant produces secondary metabolites which have the potential to be developed as medicinal raw material compounds.

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1. INTRODUCTION

High drug prices are still a health problem in Indonesia [1]. The high price of medicines is caused by the raw materials for medicines having to be imported from abroad [2]. Apart from price, drug resistance is also a problem, along with the increasing use of antibiotics [3]. Such a large role in medicine demands alternative sources of medicinal raw materials that are affordable and safe to use. Medicinal raw materials can be obtained from nature, such as plants or marine life [4]. But each has a weakness. The use of raw materials from plants requires large amounts of plant biomass and a long waiting time to harvest it. Taking plants from the forest can lead to plant extinction. Meanwhile, marine biota such as algae and soft corals are difficult to obtain in large enough quantities [4].

This situation has triggered the exploration of natural resources as a source of raw materials for medicines [5], which are more effective and have lower toxicity [6]. Natural medicinal raw materials can be obtained from bioactive compounds from nature [4], such as microbes. One of the microorganisms that produce bioactive compounds is endophytic microbes, microbes that reside in living plant tissue without harming the host plant [4]. Endophytic microbes have economic significance because microorganisms are organisms that are easy to grow, have a short life cycle, and can produce bioactive compounds quickly [7].

Microorganisms, in this case, endophytic microbes, need to be explored and developed, considering that the need for medicinal raw materials is increasing both in number and type [8]. Attention to endophytic microbes has increased in recent years because they have several functions, including being able to produce secondary metabolites in the form of compounds that have the potential to be anticancer, antiviral, antibacterial, anti-fungus, anti-diabetic, and so on [9]. Endophytic microbes can be found in plant tissues such as seeds, leaves, flowers, fruits, twigs, stems, and roots [10]. The relationship between endophytic microbes and host plants is a form of symbiotic mutualism, which is a form of mutually beneficial relationship. Endophytic microbes obtain nutrients from the host plant's body, whereas host plants obtain protection against pathogens from compounds produced by endophytic microbes [11].

Endophytic microbes can produce the same bioactive compounds as their host plants. This occurs because of the possibility of genetic transfer between host plants and endophytic microbes so that beneficial substances in plants can also be produced by their endophytic microbes [12]. If endophytic microbes are isolated from medicinal plants, the resulting secondary metabolites are the same as those contained in their host plants. This is an opportunity to obtain natural, cheap, and environmentally friendly sources of medicinal ingredients [13]. By utilizing endophytic microbes, there is no need to use large amounts of plant parts or marine biota. The development of endophytic microbes not only enables the discovery and production of natural medicinal ingredients in a sustainable and environmentally friendly manner but also opens opportunities for the discovery of other bioactive secondary metabolites which so far have often been neglected to one day be produced on a commercial scale [4].

Endophytic microbes in plant parts can consist of several types of microbes, one of which is most frequently isolated, namely endophytic fungi [4]. Endophytic fungus are the most common group of endophytic microbes found and occur in large numbers in nature [8]. The magnitude of this estimate is because one plant species can be colonized by one or several endophytic fungi [14]. Endophytic fungi are reported to produce various bioactive metabolites, both known compounds and new compounds [15]. Several studies have reported success in isolating endophytic fungus from various plants, including *Syzygium cumini* Linn [16], *Vitex trifolia* L. [17], *Begonia medicinalis* [18].

To obtain secondary metabolite bioactive compounds from endophytic funguses, one approach that can be used is to select host plants that are known to have the bioactivity in question. Several types of plants, especially medicinal plants, can be used as sources of endophytic fungi isolates. One type of plant that has the potential as a medicinal plant is *Imperata cylindrica* (L.) Beauv. Previous research has investigated the benefits of *Imperata cylindrica* (L.) Beauv. [19], including as antibacterial [20] and antioxidants [21].

In previous research, endophytic fungi was isolated from *Imperata cylindrica* (L.) Beauv. plants [22] and its potential as an antibacterial was known [6]. Isolation of the endophytic fungi was carried out in dry environmental conditions. Many research results show that different environmental conditions can exhibit different types of endophytic microbes and chemical compounds. Therefore, this research was carried out to isolate endophytic fungus from *Imperata cylindrica* (L.) Beauv. plants which grow in high rainfall conditions as well as testing the phytochemicals of endophytic fungus to determine the type of secondary metabolites produced. With this research, it is hoped that we will be able to identify the types of secondary metabolites produced by endophytic fungi from *Imperata* plants, which will enable them to be developed into medicines, thus providing an opportunity for the

pharmaceutical industry to develop medicinal raw materials that utilize the natural riches of Indonesia's land.

2. RESEARCH METHOD

Materials. The materials used include *Imperata cylindrica* (L.) Beauv. plants, PDA media (*Potato Dextrose Agar*), PDB media (*Potato Dextrose Broth*), detergent, 1% hypochlorite solution, 96% alcohol, chloramphenicol, lactophenol cotton blue (LPCB), spirits, alcohol 70%, distilled water, tissue, glycerol, glacial acetic acid, Mayer's reagent, Dragendorff's reagent, FeCl₃ solution, Mg ribbon, concentrated HCl, H₂SO₄, chloroform, gelatin 10%, concentrated hydrochloric acid magnesium powder, Lieberman-Burchard reagent, anhydrous acetic acid, sulfuric acid. Equipment used includes autoclave, laminar air flow, incubator, water bath shaker, hot plate, magnetic stirrer, vortex, set of glassware, object glass and cover glass, tweezers, alcohol bottle, knife, plastic wrap, aluminum foil, sterile cotton, a paper wrapper, test tube, micropipette, and tip, label paper, marker, scissors and ruler, bunsen, volume pipette, drop pipette, petri dish, test tube rack, beaker, measuring cup, glass funnel, measuring flask, measuring cup.

The research procedure was carried out through several stages, namely sampling, isolation, and purification of endophytic fungus, identification of endophytic fungus, production of secondary metabolites of endophytic fungi, and screening of fermented phytochemicals of endophytic fungi.

Sampling. Sampling was carried out by taking all parts of the plant from the *Imperata* fields. *Imperata cylindrica* (L.) Beauv. plants are selected that are healthy, not wilted, and have no signs of plant disease attack, then stored in an ice box and brought to the laboratory to isolate the endophytic fungi.

Endophytic Fungi Isolation. Isolation of endophytic fungus from *Imperata cylindrica* (L.) Beauv. plants begins with washing the roots, stalks, leaves, and flowers of the plants with running water and soaking them in detergent water, which is continued in 5.25% hypochlorite solution for 1 minute, in 96% alcohol for 30 seconds, rinsed with sterile distilled water three times. Isolation of endophytic fungi was carried out using the direct planting method. Parts of the *Imperata cylindrica* (L.) Beauv. plants were cut measuring 1 cm × 2 cm using a sterile knife. The *Imperata cylindrica* (L.) Beauv. plant cuttings were inoculated into PDA media in a petri dish. Then incubate for 3-7 days at room temperature [23].

Purification of Endophytic Fungi Isolates. Endophytic fungus that have grown on PDA isolation media are then gradually purified one by one. Each pure isolate of endophytic fungi obtained was then transferred to PDA media. This purification aims to separate endophyte colonies with different morphologies to become separate isolates. Morphological observations were carried out again after incubation for 3-7 days, and if macroscopically different colony growth was still found, they were separated again until pure isolates were obtained [22], [23]. **Identification of Endophytic Fungus.** Isolates were identified based on their macroscopic and microscopic characteristics. Macroscopic observations based on fungi morphology include color and colony surface. Microscopic observations based on observations of preparations include the shape of the hyphae and the shape of the reproductive cells. All observations were then compared with the literature to find out the identity of the fungi [22].

Secondary Metabolite Production of Endophytic Fungi. Purified endophytic fungi isolates were grown in PDA media for 5 days until they were sporulated. Using the pure isolate, take 5 pieces with a size of 1 × 1 cm and put them in an Erlenmeyer containing 40 ml of PDB. The Erlenmeyer was incubated in a shaker incubator at room temperature (27 – 30°C), shaking at 120 rpm for 14 days, then 20 ml of starter medium was taken and put into an Erlenmeyer containing 200 ml of PDB media. After that, fermentation was carried out, shaking at a speed of 120 rpm at room temperature (27 – 30°C). On the 9th day, the fermentation results were taken and centrifuged at 3000 rpm for 10 minutes at room temperature. The supernatant is separated from the biomass [6], [24], [23].

Phytochemical Screening of Endophytic Fungi Fermentation Results. 1). Alkaloid Test. The sample is added with dilute ammonia, chloroform is added and filtered. The filtrate was added with H₂SO₄ and homogenized. Separate the acid (top) and chloroform (bottom) layers. The acid layer was divided into two parts, each given Mayer's reagent and Dragendorff's reagent. A positive result

forms a white precipitate with Mayer's reagent and a positive result for Dragendorff if an orange-brown precipitate forms [25] 2). **Saponin Test.** The sample was added with 10 mL of hot water and then cooled. The test solution was shaken vertically for 10 seconds and then allowed to stand for 10 minutes. 1 to 10 cm high froth is formed. On the addition of 1 drop of HCl 2 N, the foam did not disappear [26] 3). **Tannin Test.** Sample added with water. Then heat until boiling for 5 minutes. The filtrate is filtered add 10% gelatin. If a white precipitate forms then the extract contains tannin [25] 4). **Phenolic Test.** The sample was dissolved in distilled water and heated. The solution is cooled and filtered. The filtrate was reacted with 1% FeCl₃. A positive result is indicated by a dark blue or blackish-green solution [25] 5). **Flavonoid Test.** The sample is dissolved in alcohol and then heated over a water bath. After that add magnesium powder and concentrated hydrochloric acid. The presence of flavonoids is indicated by the formation of red, yellow, or orange colors [26]. 6). **Terpenoid and Steroid Test.** The sample was dissolved in chloroform and then dried in a water bath. The residue obtained was added with Lieberman-Burchard reagent. A positive result of the terpenoid solution will be red, and the green-blue color is steroids [25]. 7). **Glycoside Test.** Testing using the Liebermann-Burchard reaction is carried out by adding 5 mL of anhydrous acetic acid P to the sample. 10 drops of sulfuric acid P are added, and a blue or green color appears indicating the presence of glycosides [23]. The data resulting from this research were analyzed descriptively and presented in the form of tables or images.

3. RESULTS AND DISCUSSIONS

This research was conducted to isolate endophytic fungi and test phytochemicals from *Imperata cylindrica* (L.) Beauv.). Phytochemical tests were carried out to determine the type of secondary metabolites produced. Imperata plant samples were obtained from the Neglasari area, Tangerang City, which grows under conditions of high rainfall. The parts of the Imperata plant used are roots, stalks, leaves, and flowers. The Imperata plants were determined to determine the correctness of the sample used. The determination was carried out at the Herbarium Bogoriense Botany, Center for Research and Development for Biology-LIPI Bogor.

The research procedure began with the isolation of endophytic fungi from parts of the Imperata plant by sterilizing it first. Sterilization before incubation is effective in killing epiphytic microbes or microbes that stick to the surface of the leaves so that the colonies that grow on the surface of the PDA agar medium are colonies of endophytic fungi from plant cuttings. Sterilization consists of several stages, namely washing with running water, soaking in 5.25% hypochlorite, soaking in 96% alcohol, and rinsing with sterile distilled water.

After sterilization, parts of the *Imperata cylindrica* (L.) Beauv.) plants were planted in PDA media that had antibiotics added to it and incubated for 7 days at room temperature. Endophytic fungus that had been incubated for 7 days were then sub-cultured to obtain pure endophytic fungi isolates. The isolated endophytic fungi isolates were then purified by inoculating them into a new PDA medium. All pure isolates of endophytic fungi obtained were identified macroscopically, which included the color and surface of the fungi colonies. Meanwhile, to confirm the differences between fungus, microscopic observations were made based on the shape of the hyphae, conidia, and conidiophores. The results of these observations were compared with the literature. From the results of this research, 7 isolates were obtained, and each isolate produced secondary metabolites as listed in Table 1.

Table 1. Isolates and Types of Secondary Metabolites of Endophytic Fungi from *Imperata cylindrica* (L.) Beauv.

Isolate Code	Origin of <i>Imperata cylindrica</i> (L.) Beauv. Parts	Types of Fungi	Types of Secondary Metabolites
Isolate A	flower	<i>Fusarium</i> sp	Flavonoids
Isolate B	stalk	<i>Mucor</i> sp.	Flavonoids
Isolate C	stalk	<i>Penicillium</i> sp.	Flavonoids
Isolate D	stalk	<i>Trichoderma</i> sp.	Flavonoids
Isolate E	root	<i>Aspergillus</i> sp.	Flavonoids
Isolate F	root	<i>Mucor</i> sp.	Flavonoids, Saponin
Isolate G	root	<i>Phytophthora</i> sp.	Flavonoids

Based on the results in Table 1., it is known that from the Imperata plant, 1 isolate was obtained from the flower part, 3 isolates from the stalk part, and 3 isolates from the root part. The number of endophytic fungus in each section is different, this is thought to be influenced by adaptation between the endophytic fungus and their hosts. The adaptability of endophytic fungi varies, this is due to the physiological factors of the host plant, which makes the number of endophytic fungus found from each part of the plant vary. The interactions that occur between endophytic fungus and their hosts affect the variety of types of endophytic fungus obtained [27]. Each isolate has macroscopic and microscopic characters as follows.



Figure 1. Macroscopic (left) and microscopic (right) of Isolate A

Isolate A in Figure 1. was obtained from the flower parts of the Imperata plant. Isolate A has macroscopic characteristics which include white surface color, cottony texture, uneven edges, raised wavy surface, and scattered colony growth pattern. Microscopic features include hyphae having walls and septa, hyalin, conidiophores having branches, and bent microconidia. Based on these characteristics and compared with the literature [3], the endophytic fungus isolates A belongs to the genus Genus *Fusarium*.



Figure 2. Macroscopic (left) and microscopic (right) of Isolate B

Isolate B in Figure 2. was obtained from the stalks of the Imperata plant. Isolate B has macroscopic characteristics which include a white surface color and greenish inside, cottony-powdery texture, flat edges, raised surface, and round colony growth pattern. Microscopic features include the presence of stolons but no rhizoids, and short sporangiophores. Based on these characteristics and compared with the literature [3], the endophytic fungus isolate B belongs to the Genus *Mucor*.



Figure 3. Macroscopic (left) and microscopic (right) of Isolate C

Isolate C in Figure 3. was obtained from the stalk of the Imperata plant. Isolate C has macroscopic characteristics which include green surface color, elliptical-shaped colonies, mostly consisting of dense

conidiophores, and velvety colonial surfaces. Microscopic characteristics include conidia in the form of long chains, divergent, globular, elliptical or fusiform, greenish, wavy walls. Based on these characteristics and compared with the literature [3], the endophytic fungus isolate C belongs to the Genus *Penicillium*.

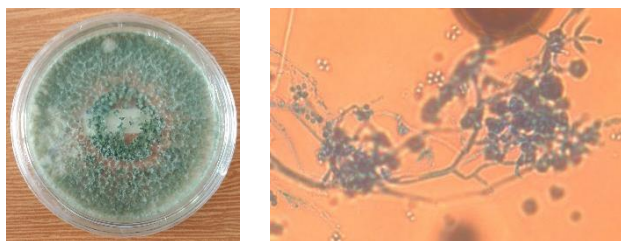


Figure 4. Macroscopic (left) and microscopic (right) of Isolate D

Isolate D in Figure 4. was obtained from the stalk of the Imperata plant. Isolate D has macroscopic characteristics which include the initial form of white colonies and with increasing age, they turn dark green, granular, and scattered colonies. Microscopic features include conidiophores that branch regularly and do not form bundles, conidia are oblong, single-celled, in small terminal groups, and conidium groups are blue-green. Based on these characteristics and compared with the literature [3], the endophytic fungus isolate D belongs to the Genus *Trichoderma*.

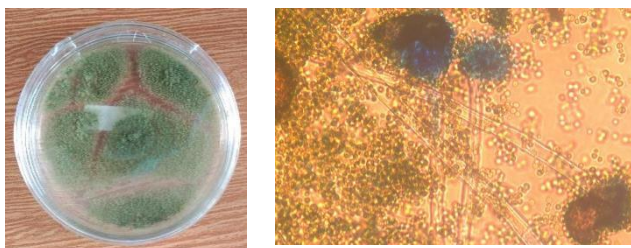


Figure 5. Macroscopic (left) and microscopic (right) of Isolate E

Isolate E in Figure 5. was obtained from the roots of the Imperata plant. Isolate E has macroscopic characteristics which include green colonies, greenish conidia with greenish spots on the surface, and concentric arrangement. Microscopic features include hyphae that are not insulated, clustered, and attached to vesicles, conidia that are abundant and round, and conidiophores are unbranched and have rough walls. Based on these characteristics and compared with the literature [3], the endophytic fungus isolate E belongs to the Genus *Aspergillus*.

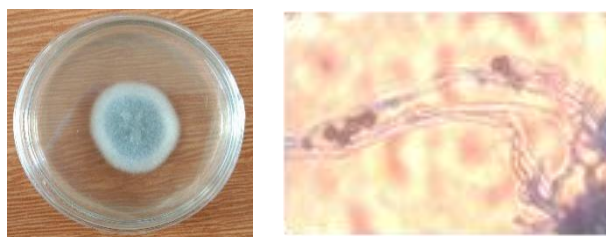


Figure 6. Macroscopic (left) and microscopic (right) of Isolate F

Isolate F in Figure 6. was obtained from the roots of the Imperata plant. Isolate F has macroscopic characteristics which include the top part of the mycelium is green-gray, the middle and the edges are white. The lower mycelium is dark green. Microscopic features include round conidia, abundant in the conidiophores, and black, and conidiophores that are unbranched and have thin walls. Based on these

characteristics and compared with the literature [3], the endophytic fungus isolate F belongs to the Genus *Mucor*.

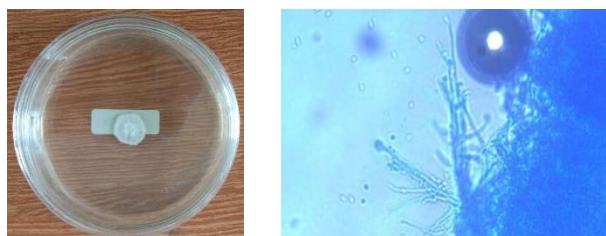


Figure 7. Macroscopic (left) and microscopic (right) of Isolate G

Isolate G in Figure 7. was obtained from the roots of the Imperata plant. Isolate G has macroscopic characteristics which include a round colony shape, white color, uneven colony edges, zoning, and radial lines on the fungi colony. Microscopic features include hyphae without septa, sporangia are ovoid, like a lemon, and have papillae. Based on these characteristics and compared with the literature [3], the endophytic fungus isolate F belongs to the Genus *Phytophthora*.

The endophytic fungi that have been isolated and identified are then fermented, and a phytochemical test is carried out to determine the type of secondary metabolite compounds produced by these endophytic fungi [28], [29]. Phytochemical tests were carried out on all endophytic fungus isolates that were isolated from Imperata plants (Table 1). Before the phytochemical tests were carried out, the endophytic fungus isolates were fermented in PDB media, to produce large amounts of endophytic fungal cells so as to optimize the resulting metabolite compounds. The fermentation process was carried out for 14 days, which is the most suitable fermentation time to produce maximum growth of fungi and metabolite production [6]. Based on the results of phytochemical tests on endophytic fungus (Table 1), it is known that all endophytic fungi isolates produce secondary metabolites. *Fusarium* sp. in Figure 1. was obtained from the flower parts of the Imperata plant, produces flavonoids. *Mucor* sp. in Figure 2. was obtained from the stalk of the Imperata plant, produces flavonoids. *Penicillium* sp. in Figure 3. was obtained from the stalk of the Imperata plant, produces flavonoids. *Trichoderma* sp. in Figure 4. was obtained from the stalk of the Imperata plant, produces flavonoids. *Aspergillus* sp. in Figure 5. was obtained from the roots of the Imperata plant, produces flavonoids. *Mucor* sp. in Figure 6. was which is obtained from the roots of the Imperata plant, produces flavonoids and saponins. *Phytophthora* sp. in Figure 7. was obtained from the roots of the Imperata plant, produces flavonoids.

The results of flavonoid secondary metabolites produced by endophytic fungi in this study are from previous research which succeeded in finding flavonoids in endophytic fungi from *Ginkgo biloba* plants [30], endophytic fungi from *Conyza blinii* plants [31], endophytic fungi from the *Loranthus tanakae* plant [32]. Meanwhile, positive results for the saponin group were shown after the sample was added with 10 mL of hot water and then cooled, the test solution was shaken vertically for 10 seconds, then allowed to stand for 10 minutes, and foam 1 to 10 cm high was formed. Positive saponin is indicated by, after adding 1 drop of 2 N HCl the foam that forms does not disappear [26]. The results of saponin-type secondary metabolites produced by endophytic fungi in this study are by previous research which succeeded in finding saponin in endophytic fungi from *Uncaria cordata* leaves [33]; endophytic fungi from mahogany trees [34], endophytic fungi from *Asparagus racemosus* [35].

The types of secondary metabolites produced by endophytic fungi isolated from Imperata plants in this study are the secondary metabolites produced by Imperata plants. Several studies state that Imperata plants contain secondary metabolites such as flavonoids, tannins, alkaloids, and saponins [36]. Added by Kusuma et.al. [6], the flavonoid content in Imperata plants is 4.8%. So the results of this study are by the statement [37], that the ability of endophytic microbes to produce secondary metabolite compounds is the same as that of their host plants.

Flavonoids are known to have antimicrobial, antiallergic, antioxidant, antitumor cytotoxic, anti-inflammatory, hypoallergenic, hepatoprotective antithrombotic, and antiviral activities [38], [38] [39]. Flavonoids also act as metal chelators and scavengers of free radicals, as well as strong antioxidants [40]. While saponins act as antimicrobial compounds. More than 90%, of the bacterial cell wall on the outer membrane is protected by lipopolysaccharide (LPS) [41]. Saponins will react with lipopolysaccharides thereby reducing the surface tension of the membrane, which will eventually cause cell destruction [42].

The ability of endophytic fungi to produce secondary metabolites similar to those of their host plants is an advantage in itself. These advantages can be seen in terms of time and cost, considering that it takes a long time and quite a lot of plant biomass to produce one type of secondary metabolite. Research conducted Jamilatun et.al. [6], shows that the endophytic fungi isolate of *Imperata cylindrica* (L.) Beauv isolates has antibacterial activity and the results of this study show that the endophytic fungi isolate of *Imperata* plat produces secondary metabolites such as flavonoids and saponins. With the presence of this potential endophytic fungi, it is hoped that both types of secondary metabolites can be produced optimally, and can be developed through further research to determine other bioactivities of secondary metabolite compounds produced by endophytic fungi isolates from the *Imperata cylindrica* (L.) Beauv.

4. CONCLUSION

There were seven isolates of endophytic fungus found in the *Imperata cylindrica* (L.) Beauv. The types of endophytic fungus consist of *Fusarium* sp. isolated from floral parts, *Mucor* sp. isolated from the stem and roots, *Penicillium* sp. and *Trichoderma* isolated from the stem, as well as *Aspergillus* sp. and *Phytophthora* sp. which was isolated from the roots of the *Imperata cylindrica* (L.) Beauv. Each endophytic fungi produces secondary metabolites such as flavonoids and saponins which are also produced by *Mucor* sp isolates. which is obtained from the roots of the *Imperata* plant. Based on the type of secondary metabolites produced by endophytic fungus from *Imperata* plants, it has the potential to be developed into medicine so that it becomes an opportunity to develop medicinal raw materials that utilize endophytic fungi isolates from *Imperata cylindrica* (L.) Beauv. In its development as a drug, the results of this research still have limitations because the secondary metabolite activity produced by each endophytic fungi isolate has not been tested. So it is necessary to carry out further research, to determine the potential of secondary metabolites of *Imperata cylindrica* (L.) Beauv plant endophytic fungi as antimicrobial, antioxidant, anticancer, antidiabetic agents, and other biological activities that play a role in drug development.

REFERENCES

- [1] L. S. Muis, "Hak Atas Aksesibilitas Obat Paten Bagi Masyarakat," *Widya Pranata Huk. J. Kaji. dan Penelit. Huk.*, vol. 1, no. 1, pp. 36–64, 2019, doi: 10.37631/widyapranata.viii.259.
- [2] Kemenperin, "Membangun Kemandirian Industri Farmasi Nasional: Buku Analisis Pembangunan Industri-Edisi II 2021," *Buku Anal. Pembang. Ind.*, pp. 1–38, 2021, [Online]. Available: <https://www.kemenperin.go.id/download/26388/Buku-Analisis-Industri-Farmasi-2021>.
- [3] Y. Suryani, O. Taupiqurrahman, and Y. Kulsum, *Mikologi*, no. 1. Padang, Sumatera Barat: PT. Freeline Cipta Granesia, 2020.
- [4] S. Kumala, *Mikroba Endofit: pemanfaatan mikroba endofit dalam bidang farmasi*, vol. 11. 2019.
- [5] A. Widyasanti, Y. Qurratu'ain, and S. Nurjanah, "Chimica et Natura Acta," *Chim. Nat. Acta*, vol. 6, no. 3, pp. 127–135, 2018, [Online]. Available: <https://google.co.id>.
- [6] M. Jamilatun, A. Aminah, and S. Shufiyani, "Uji Daya Hambat Antibakteri Kapang Endofit dari Tanaman Alang-Alang (*Imperata cylindrica* (L.) BEAUV.) terhadap Pertumbuhan Bakteri *Staphylococcus aureus* dan *Escherichia coli*," *J. Med. (Media Inf. Kesehatan)*, vol. 7, no. 2, 2020, doi: 10.36743/medikes.v7i2.224.
- [7] A. Tripathi, P. Pandey, S. N. Tripathi, and A. Kalra, "Perspectives and potential applications of endophytic microorganisms in cultivation of medicinal and aromatic plants," *Frontiers in Plant Science*, vol. 13. 2022, doi: 10.3389/fpls.2022.985429.
- [8] M. M. Gakuubi, M. Munusamy, Z. X. Liang, and S. B. Ng, "Fungal endophytes: A promising frontier for discovery of novel bioactive compounds," *J. Fungi*, vol. 7, no. 10, 2021, doi: 10.3390/jof7100786.

- [9] A. H. Hashem *et al.*, "Bioactive compounds and biomedical applications of endophytic fungi: a recent review," *Microbial Cell Factories*, vol. 22, no. 1, 2023, doi: 10.1186/s12934-023-02118-x.
- [10] O. I. Omomowo and O. O. Babalola, "Bacterial and fungal endophytes: Tiny giants with immense beneficial potential for plant growth and sustainable agricultural productivity," *Microorganisms*, vol. 7, no. 11, 2019, doi: 10.3390/microorganisms7110481.
- [11] P. Jha *et al.*, "Endophytic fungi: hidden treasure chest of antimicrobial metabolites interrelationship of endophytes and metabolites," *Frontiers in Microbiology*, vol. 14, 2023, doi: 10.3389/fmicb.2023.1227830.
- [12] T.-D. Nguyen *et al.*, "Relationships between endophytic bacteria and medicinal plants on bioactive compounds production," *Rhizosphere*, vol. 27, 2023, doi: 10.1016/j.rhisph.2023.100720.
- [13] C. E. Ezeobiora, N. H. Igbokwe, D. H. Amin, and U. E. Mendie, "Endophytic microbes from Nigerian ethnomedicinal plants: a potential source for bioactive secondary metabolites—a review," *Bull. Natl. Res. Cent.*, vol. 45, no. 1, 2021, doi: 10.1186/s42269-021-00561-7.
- [14] J. B. A. dos Reis, A. S. Lorenzi, and H. M. M. do Vale, "Methods used for the study of endophytic fungi: a review on methodologies and challenges, and associated tips," *Archives of Microbiology*, vol. 204, no. 11, 2022, doi: 10.1007/s00203-022-03283-0.
- [15] D. C. Fontana *et al.*, "Endophytic fungi: Biological control and induced resistance to phytopathogens and abiotic stresses," *Pathogens*, vol. 10, no. 5, 2021, doi: 10.3390/pathogens10050570.
- [16] M. M. S. Samapti *et al.*, "Isolation and Identification of Endophytic Fungi from *Syzygium cumini* Linn and Investigation of Their Pharmacological Activities," *Sci. World J.*, vol. 2022, 2022, doi: 10.1155/2022/9529665.
- [17] M. Hasan Basri, L. Zulkifli, and A. Syukur, "Isolation of Endophytic Fungi from *Vitex trifolia* L and Antagonism Test against *Sclerotium rolfii* and pathogenic bacteria," *J. Biol. Trop.*, vol. 21, no. 1, 2021, doi: 10.29303/jbt.v21i1.2340.
- [18] S. Anam, A. Syamsidi, M. Musyahidah, N. Ambianti, A. Widodo, and M. S. Zubair, "Isolation of endophytic fungi from benalu batu (*Begonia Medicinalis*) and their toxicity on *Artemia Salina*," *J. Ilm. Farm.*, 2022, doi: 10.20885/jif.specialissue2022.art3.
- [19] Z. Zulkarnain, E. Wijayanti, U. Fitriani, and A. Triyono, "Studi Literatur untuk Memperoleh Dasar Ilmiah Penggunaan Akar Alang-alang sebagai Ramuan Jamu untuk Penyembuhan Beberapa Penyakit di Rumah Riset Jamu Hortus Medicus," *Media Penelit. dan Pengemb. Kesehat.*, vol. 29, no. 4, 2020, doi: 10.22435/mpk.v29i4.2105.
- [20] Y. K. Jung and D. Shin, "Imperata cylindrica: A review of phytochemistry, pharmacology, and industrial applications," *Molecules*, vol. 26, no. 5, 2021, doi: 10.3390/molecules26051454.
- [21] D. Dhianawaty, R. Ruslin, M. R. A. A. Syamsunarno, and H. Haminah, "Kandungan Total Flavonoid Dari Ekstrak Metanol Akar Imperata cylindrical (L) Beauv. (Alang-alang)," *Talent. Conf. Ser. Trop. Med.*, vol. 1, no. 3, 2018, doi: 10.32734/tm.v1i3.256.
- [22] M. Jamilatun and S. Shufiyani, "Isolasi dan Identifikasi Kapang Endofit dari Tanaman Alang-Alang (*Imperata cylindrica* (L.) BEAUV.)," *J. Med. (Media Inf. Kesehatan)*, vol. 6, no. 1, 2019, doi: 10.36743/medikes.v6i1.92.
- [23] W. S. Abdulkadir, M. S. Pakaya, and F. N. Ramadhani, "Analisis Kualitatif Metabolit Sekunder dan Aktivitas Antioksidan Jamur Endofit Cangkang Bulu Babi (*Diadema setosum*)," vol. 3, no. 2, pp. 280–290, 2023, doi: 10.37311/ijpe.v3i1.18063.
- [24] W. A. Mahardhika, M. G. Isworo Rukmi, and S. Pujiyanto, "Isolasi kapang endofit dari tanaman ciplukan (*Physalis angulata* L.) dan potensi antibakteri terhadap *Escherichia coli* dan *Staphylococcus aureus*," *NICHE J. Trop. Biol.*, vol. 4, no. 1, 2021.
- [25] I. Sulistyarni, D. A. Sari, and T. A. Wicaksono, "Skrining Fitokimia Senyawa Metabolit Sekunder Batang Buah Naga (*Hylocereus polyrhizus*)," *J. Ilm. Cendekia Eksakta*, pp. 56–62, 2019.
- [26] D. Devi, A. Anggraeni, and T. Wahyuni, "Isolasi Kapang Endofit Pelawan (*Tristaniopsis merguensis* Griff.) yang Berpotensi Sebagai Antibakteri Terhadap *Escherichia coli* dan *Staphylococcus aureus*," *Al-Kaunyah J. Biol.*, vol. 14, no. 2, pp. 195–206, 2021, doi: 10.15408/kaunyah.v14i2.14051.
- [27] B. Alam *et al.*, "Endophytic Fungi: From Symbiosis to Secondary Metabolite Communications or Vice Versa?," *Frontiers in Plant Science*, vol. 12, 2021, doi: 10.3389/fpls.2021.791033.
- [28] T. S. Julianto, *Fitokimia Tinjauan Metabolit Sekunder dan Skrining fitokimia*, vol. 53, no. 9, 2019.
- [29] B. Ma'arif, A. Mahardiani, and D. M. Mirza, "Fitokimia dan Aplikasinya," *Penerbit Sintesa Books*, pp. 12–26, 2021.
- [30] X. Y. Zhang *et al.*, "Separating and Purifying of Endophytic Fungi from Ginkgo Biloba and Screening of Flavonoid-Producing Strains," in *IOP Conference Series: Earth and Environmental Science*, 2019, vol. 371, no. 4, doi: 10.1088/1755-1315/371/4/042052.
- [31] Z. Tang *et al.*, "Isolation and identification of flavonoid-producing endophytic fungi from medicinal plant

- Conyza blinii* H.Lév that exhibit higher antioxidant and antibacterial activities," *PeerJ*, vol. 2020, no. 4, 2020, doi: 10.7717/peerj.8978.
- [32] Z. Zheng, S. Chai, J. Chen, H. Yang, J. Chang, and G. Yang, "Isolation and identification of flavonoid-producing endophytic fungi from *Loranthus tanakae* Franch. & Sav that exhibit antioxidant and antibacterial activities," *J. Appl. Microbiol.*, vol. 133, no. 3, 2022, doi: 10.1111/jam.15696.
- [33] M. Octaviani, W. Y. Ameliah, N. Frimayanti, M. Djohari, and H. Fadhli, "Isolation of Endophytic Fungus from Leaves of *Uncaria cordata* (Lour.) Merr and Antibacterial Activity Against *Propionibacterium acnes* and *Escherichia coli*," *Borneo J. Pharm.*, vol. 5, no. 3, pp. 279–287, 2022, doi: 10.33084/bjop.v5i3.3692.
- [34] U. S. Hastuti, S. Sulisetijono, C. Thoyibah, S. H. Pratiwi, and K. Khotimah, "Histological Observation, Identification, and Secondary Metabolites Content in Endophytic Fungi of Mahogany Tree (*Swietenia mahagoni* Jacq)," *Proc. 7th Int. Conf. Biol. Sci. (ICBS 2021)*, vol. 22, no. Icbs 2021, pp. 156–164, 2022, doi: 10.2991/absr.k.220406.024.
- [35] M. Rani, S. Jaglan, V. Beniwal, and V. Chhokar, "Bioactive saponin profiling of endophytic fungi from *Asparagus racemosus*," *Nat. Prod. Res.*, 2022, doi: 10.1080/14786419.2022.2156997.
- [36] P. B. Lalthanpuui, Zarzokimi, and K. Lalchandama, "Some pharmacognostic studies of the cogon grass *Imperata cylindrica* from Mizoram, India," *Sci. Vis.*, vol. 18, no. 3, 2018, doi: 10.33493/scivis.18.03.03.
- [37] S. STikole, D. Tarate, and P. Shelar, "Endophytic Micro-Organisms as Emerging Trend of Secondary Metabolite," *Int. J. Pharma Sci. Res.*, vol. 9, no. 12, 2018.
- [38] Prithviraj Karak, "Biological Activities of Flavonoids: an Overview," *Int. J. Pharm. Sci. Res.*, vol. 10, no. 4, pp. 1567–1574, 2019, doi: 10.13040/IJPSR.0975-8232.10(4).1567-74.
- [39] A. Ullah *et al.*, "Important flavonoids and their role as a therapeutic agent," *Molecules*, vol. 25, no. 22, 2020, doi: 10.3390/molecules25225243.
- [40] L. Khandokar, M. S. Bari, V. Seidel, and M. A. Haque, "Ethnomedicinal uses, phytochemistry, pharmacological activities and toxicological profile of *Glycosmis pentaphylla* (Retz.) DC.: A review," *Journal of Ethnopharmacology*, vol. 278, 2021, doi: 10.1016/j.jep.2021.114313.
- [41] I. I. Tatli Cankaya and E. I. Somuncuoglu, "Potential and Prophylactic Use of Plants Containing Saponin-Type Compounds as Antibiofilm Agents against Respiratory Tract Infections," *Evidence-based Complementary and Alternative Medicine*, vol. 2021, 2021, doi: 10.1155/2021/6814215.
- [42] S. M. Astari, A. Rialita, and M. Mahyarudin, "Aktivitas Antibakteri Isolat Bakteri Endofit Tanaman Kunyit (*Curcuma longa* L.) Terhadap Pertumbuhan *Staphylococcus aureus*," *J. Fitofarmaka Indones.*, vol. 8, no. 2, pp. 9–16, 2021, doi: 10.33096/jffi.v8i2.644.