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ACTIVITY OF ENDOFIT MOLD METABOLITES INSULATED FROM BETEL LEAVES (Piper betle L.) On FUNGUS *Candida albicans*

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Abstract

Betel (Piper betle) is a type of vines that have been long known and are used hereditary for the treatment. Fungal endophyte betel leaf producing antimicrobial substances and secondary metabolites that are used as the discovery, and production of new drugs. Candidiasis is a disease caused by Candida albicans when immune dysfunction occurs and causes diseases of the oral mucosa, the digestive tract and vagina. The use antifungal drugs from chemicals to cause side effects on candidates that many alternative treatments using natural ingredients as an antimicrobial. This research aims to isolate fungal endophyte of betel leaf (Piper betle L.) and know the activity of metabolites against the fungus Candida albicans. This research was conducted in the laboratory of Plant Physiology and Biotechnology, Faculty of agriculture, University of Mataram in May to June 2019. The research method used is Descriptive Observation. Research done by the fungal endophyte isolate of betel leaf and identify it. Samples of the betel leaves are obtained from the village of Bonjeruk, Kec. Jonggat, Kab. Central Lombok. Production of secondary metabolites of fungal endophyte was obtained with the method of fermentation and tested its activity against Candida albicans with the well diffusion method. The test germ used is obtained from the Litbangkes Laboratory of the NTB Province Hospital. The research results showed that fungal endophyte isolated from the leaves of the betel (Piper betle L.) and obtained the results of the identification of the fungal genus Cephalosporium sp and showed an inhibition zone of 30.9 mm.

Keyword: *Fungal Endophyte, Betel Leaf (Piper betle L.), Candida albicans*

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Introduction

Betel (Piper betle) is a type of vines that has long been known and used for generations for the treatment of coughs, toothaches, refresher and so on. Parts of betel plants such as roots, seeds and leaves have the potential for treatment, but the most often used is part of the leaves. The use of betel as a traditional treatment is due to the presence of a number of chemicals that have activity as antiplatelet, anti-inflammatory and antimicrobial compounds
(Sharma et al., 2009)

Endophytes are microorganisms that grow in plant tissues, bioactive compounds as secondary metabolites which have power as antimicrobial, antimalarial, anticancer and so on. Endophytic mushrooms are one type of endophytic microbes that live intracellular in healthy plant tissue. Some endophytes are proven to produce natural compounds that are characteristic for their hosts. The ability of endophytic microbes to produce secondary metabolites in accordance with their host plants is a huge and reliable opportunity to produce secondary metabolites from endophytic microbes isolated from these host plants (Tan & Zou, 2001). Candida albicans is a normal flora that lives on the oral mucosa, digestive tract and vagina (Sardi, Scorzoni, Bernardi, Fusco-Almeida, & Mendes Giannini, 2013). As a normal flora, Candida is not infectious, but if there is an impairment of immunity or immune dysfunction, both alone and caused by other diseases. Vaginal infections and oral candidiasis are estimated to occur as many as 40 million infections per year. The use of antifungal drugs made from chemicals such as amphotericin B, nystatin, ketoconazole, fukanazol and griseofulvin often lead to serious side effects and resistance to candidiasis(Naglik, Richardson, & Moyes, 2014).

The use of betel leaf extract (Piper betle L.) with a concentration of 80% and 100% has been shown to greatly influence the growth of candida albicans (Rahmah, Rahman, Perikanan dan Kelautan Pemerintahan Daerah Propinsi Kalimantan Selatan, & Studi Biologi Fakultas MIPA UNLAM Banjarbaru Kalimantan Selatan, 2010)
The ability of endophytic microbes to produce various phytochemical compounds is also produced by plants. Based on the background description, it is necessary to do a research on the metabolic activity test of Endophytic Fungi isolated from Betel Leaves (Piper betle) against Candida albicans fungus, considering that endophytic fungi based on literatul studies have the potential to produce secondary metabolites (Tan & Zou, 2001).

Method

The research design used in the study was a descriptive observational study conducted by isolating endophytic fungi from betel leaves (Piper betle L.) then identified and conducted by testing the endophytic fungal metabolite activity against Candida albicans. This research was conducted at the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, University of Mataram. The research was carried out in May 2019. Samples taken were leaves from betel plants (Piper betle L.) ..

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Endophytic mushrooms were isolated from betel leaves (Piper betle L.) The betel leaves were cut ± 2 cm long and washed with running water for 5 minutes. After washing, surface sterilization is done by entering into 70% alcohol solution for 5 minutes, followed by 1% NaOCl solution for 5 minutes then drying with sterile tissue. Sterile betel leaf (Piper betle L.) leaves are planted into a petri dish containing PDAC media. Observation is carried out every day until the fungus grows, then the endophytic fungus that grows is isolated and purified on a new PDAC medium. Each colony of different shapes and the color is subculture again on a new PDAC medium.

Endophytic fungi that have been incubated are then identified based on macroscopic and microscopic features. Observation of macroscopic features is done by looking directly at the shape and color of endophytic fungal colonies while microscopically observing morphological features using a microscope.

a. Procedure for testing endophytic fungi against Candida albicans

Endophytic fungi fermentation is done by using PDB (Potato Dextrose Broth) media, which aims to obtain extracts containing secondary metabolite compounds from endophytic fungi isolates. Pure endophytic fungi colony that has been sporulated on PDA media, then cut and taken 3 pieces measuring ± 1 x 1 cm. The pieces of fungi are then inoculated into a liquid fermentation medium of PDB of 500 mL. Erlenmeyer flasks containing PDB liquid fermentation media and pieces of endophytic fungal culture fermented rocking using a rotary shaker at 140 rpm (whisk / minute), carried out at room temperature (27 ° C) for 14 days (Sinaga et al., 2009; Rollando, 2009 2016). To test the activity of secondary metabolites of endophytic fungi on the growth of the fungus Candida albicans was carried out using the welling method on PDA (Potato Dextrose Agar) media by taking fermentation results of 50 µl.

Result

Growth of betel leaf endophytic fungi colonies (Piper betle L.) from isolates on PDAC medium. After isolation of endophytic fungi that grew from the betel leaf tissue (Piper betle L.) on the PDAC medium for 5 days was obtained endophytic fungi isolates with the same colony color. The image of endophytic fungus isolation from betel leaves (Piper betle L.) on PDAC medium can be seen in the picture.

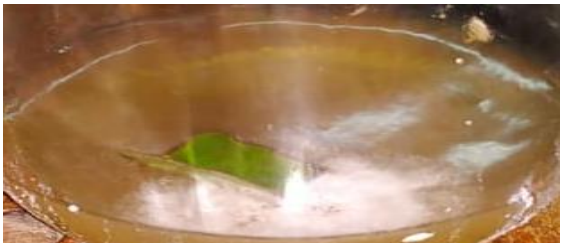

Figure 4.1 Results of isolation of betel leaf endophytic fungi (Piper betle L.) on the medium
PDAC for 5 days

The results of macroscopic observation of the appearance of endophytic fungal isolates can be seen that the isolates showed the same shape and color of the colony taken from the same sample. After purification based on the shape and color macroscopically on the new PDAC media, it was obtained the results of isolates with the same colony color.

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a. The identification of betel leaf endophytic fungi isolates (Piper betle L.) on PDAC medium

Based on observations, endophytic fungi that have been isolated and purified from betel leaves (Piper betle L.) can be identified by looking at macroscopic and microscopic characteristics, with reference to the classification guide according to Barnet (1972).

In the PDAC medium the pure white fungus colony, mycelium spreads, the growth of the colony is flat, thin as coarse velvet. It grows at room temperature, starts to grow on the PDAC medium on the fifth day. While short conidiophores, hyaline, aseptate and slim.

The results of macroscopic observations include the color of the colony, the shape of the colony, the texture of the colony and the pattern of distribution. While microscopic observations include hyphal structures (insulated or non-insulated), conidia and spores, presented in Table 4.1.

Tabel 4.1 Pengamatan makroskopis dan mikroskopis jamur endofit

Kode Isolat	Makroskopis				Mikroskopis			Jenis jamur endofit
	Koloni				Hifa	Konidia	Konidiofor	
	Warna	Bentuk	Tekstur	Pola Penyebaran				
J_1	Putih bersih	Bulat	Kasar	Beludru	Tidak bersekat	Pendek	Ramping	*Cephalosporium sp.*

a. Hasil uji aktivitas metabolit jamur endofit daun sirih (*Piper betle* L.) terhadap *Candida albicans*

Figure 4.3 Test results of endophytic fungal metabolite activity of betel leaf against Candida albicans

The results of the metabolic activity test of betel leaf endophytic fungi (Piper betle L.) showed that the isolate could inhibit the growth of Candida albicans with inhibition zone diameter reaching 30.9 mm.

Table 4.2 Observation of the test activity of endophytic fungal metabolites of betel leaf

Isolation Codes	Inhibitory power
J1	Inhibits (30.9 mm)
Control positive (+)	Inhibits (50 mm)

Based on table 4.2 the measurement results of Candida albicans growth inhibition zone diameter obtained inhibition zone diameter of 30.9 mm, the diameter of the inhibitory zone produced is included in the strong criteria (Pfaller et al., 2004)

Discussion

Based on macroscopic and microscopic observations of the betel leaf (Piper betle L.) endophytic fungus in the study (table 4.1) it is known to have macroscopic features of a clean white fungus colony, mycelium spreading, growth of flat, thin colonies such

Cephalosporium sp mushroom has the characteristics of branched mycelium, hyphae aseptates and hyaline while conidiophores are short, hyaline, aseptate and slender as well as hyaline conidia and 1 cell (Haniah, 2008)

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Moreover, according to Barnet and Hunter (1972) in (Hutabalian, Iskandar Pinem, & Oemry, 2015) the fungus Cephalosporium sp has the shape of conidiophores and fialids which are slim or slightly swollen. Conidia are transparent and conidia consist of 1 cell.

According to (Haniah, 2008) macroscopic observation of the appearance of endophytic fungal isolates of betel leaf fungi (Piper betle L.) showed a different appearance although taken on samples of betel leaves of the same type and taken from different regions and obtained nine endophytic fungal isolates, two of which were endophytic fungi included in the genus Cephalosporium sp. This is consistent with the statement (Atmosukarto, 2006) that one plant is certainly different from other plants, the biotope where microbes live is very unique in nature. In fact, the physiology of tall plants including those from the same species will be different in different environments

Based on the test results of the activity of endophytic fungal metabolites of betel leaf against Candida albicans (table 4.2) is known to be able to inhibit the growth of Candida albicans with inhibition zone diameter of 30.9 mm which is included in the strong criteria. This is in accordance with the antifungal strength criteria set by the NCCLS (Pfaller et al., 2004) which states that the antifungal strength is included in the criteria of strong if the diameter is ≥ 28 mm, the criteria are moderate if the diameter is 21-27 mm and the criteria are weak if the diameter is ≤ 20 mm

The results of this study are also strengthened by research(Haniah, 2008). Some studies state that green betel leaf (Piper betle L.) in the form of stew, juice, infusion, essential oil and ethanol extract has an antifungal effect on Candida albicans. (Soemiati & Elya, 2010)which states that endophytic fungi are able to produce antifungal, antibacterial, plant growth hormone, insecticide, etc.

The presence of antifungal activity against Candida albicans caused by endophytic fungi found in this study (table 4.1) is Cephalosporium sp. According to Kelvin (1989) the fungus genus Cephalosporium sp is able to produce cephalosporin antibiotics. Hasil penelitian(Rahmah et al., 2010).Hasil penelitian (Fridayanti, Ibrahim, & Fitriyanti, 2015) menyatakan bahwa hasil isolasi endifit dari Daun Yakon (Smallanthus sonchifolius) telah diketahui memiliki aktivitas sebaga anti jamur terhadap candida albicansa.. The discovery of differences in the amount of inhibitory zones formed may be due to the different secondary metabolites produced by endophytic fungi on betel leaves (Piper betle L.). whereas betel leaf endophytic fungi contain secondary metabolites such as Penicillin, cephalosporin, fumigasin, javanisin and chetomin (Hutabalian et al., 2015)

In addition to sepalosporin compounds contained in betel leaves, betel leaf endophytic fungi also produce phenyl propane compounds (phenolic compounds (Rahmah et al., 2010). These phenolic compounds can cause denaturation of proteins, namely damage to tertiary protein structures

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making up the fungal cell walls so that it will result in weakness function of other microorganism cell wall proteins Secondary metabolites of endophytic fungi found in betel leaves (Piper betle L.) can be influenced by the temperature factor of the betel plant to grow, the more humid the environment the better the metabolite compounds produced by betel plants. in accordance with the results of research conducted by(Budiprakoso, 2010) that the abundance of endophytic fungi is influenced by biotic and abiotic factors.Biotic factors consist of varieties and host species, while the abiotic factors that influence are weather factors namely temperature, relative humidity and soil water content and cultivation techniques This is supported by previous research (Uma Maheswari & Saranya, 2018) that endophytic fungi that live in healthy plant tissues are able to produce the same bioactive metabolites or their derivatives which can be more active than those produced by their host plants.

Based on the results of research that has been done, the following conclusions are obtained:

a. Endophytic fungi were successfully isolated from betel leaves (Piper betle L.) by using samples from Bonjeruk Village, Kec. Jonggat, Kab. Central Lombok, West Nusa Tenggara. The results of identification of endophytic fungi based on macroscopic and microscopic features obtained from endophytic fungi from the genus Cephalosporium sp.

b. The results of the endophytic fungal metabolite activity test showed that the isolate can inhibit the growth of Candida albicans with inhibition zone diameter of 30.9 mm included in the strong criteria.

REFERENCES

Atmosukarto, A. P. dan I. (2006). *Mikroba Endofit: Sumber Molekul Acuan Baru yang Berpotensi* (pp. 13–15). pp. 13–15.

Budiprakoso, B. (2010). *Pemanfaatan Cendawan Endofit sebagai Penginduksi Ketahanan Tanaman Padi terhadap Wereng Coklat Nilaparvata lugens (Stal). (Hemiptera: Delphacidae)*.

Fridayanti, A., Ibrahim, A., & Fitriyanti. (2015). Aktivitas antijamur dan identifikasi metabolit sekunder isolat jamur endofit dari daun yakon. *J.Trop.Pharm.Chem*, *3*(2), 88–93.

Haniah, M. (2008). Isolasi jamur endofit dari daun sirih (. *Repository Universitas Islam Negeri Malang*.

Hutabalian, M., Iskandar Pinem, M., & Oemry, S. (2015). Uji Antagonisme Beberapa Jamur Saprofit Dan Endofit Dari Tanaman Pisang Terhadap Fusarium Oxysporum F.sp. Cubens Di Laboratorium. *Jurnal Agroekoteknologi Universitas Sumatera Utara*, *3*(2), 687–695. https://doi.org/10.32734/jaet.v3i2.10354

Naglik, J. R., Richardson, J. P., & Moyes, D. L. (2014). Candida albicans Pathogenicity and Epithelial Immunity. *PLoS Pathogens*, *10*(8), 8–11. https://doi.org/10.1371/journal.ppat.1004257

ISSN-e: 2541-1128
ISSN-p: 2407-8603

Pfaller, M. A., Messer, S. A., Boyken, L., Rice, C., Tendolkar, S., Hollis, R. J., & Diekema, D. J. (2004). Evaluation of the NCCLS M44-P Disk Diffusion Method for Determining Susceptibilities of 276 Clinical Isolates of Cryptococcus neoformans to Fluconazole. *Journal of Clinical Microbiology*, *42*(1), 380–383. https://doi.org/10.1128/JCM.42.1.380-383.2004

Rahmah, N., Rahman, A. K., Perikanan dan Kelautan Pemerintahan Daerah Propinsi Kalimantan Selatan, D., & Studi Biologi Fakultas MIPA UNLAM Banjarbaru Kalimantan Selatan, P. (2010). UJI FUNGISTATIK EKSTRAK DAUN SIRIH (Piper betle L.) TERHADAP Candida albicans. *Bioscientiae*, *7*(2), 17–24.

Sardi, J. C. O., Scorzoni, L., Bernardi, T., Fusco-Almeida, A. M., & Mendes Giannini, M. J. S. (2013). Candida species: Current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *Journal of Medical Microbiology*, *62*(PART1), 10–24. https://doi.org/10.1099/jmm.0.045054-0

Sharma, S., Khan, I. A., Ali, I., Ali, F., Kumar, M., Kumar, A., … Qazi, G. N. (2009). Evaluation of the antimicrobial, antioxidant, and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. *Antimicrobial Agents and Chemotherapy*, *53*(1), 216–222. https://doi.org/10.1128/AAC.00045-08

Soemiati, A., & Elya, B. (2010). UJI PENDAHULUAN EFEK KOMBINASI ANTIJAMUR INFUS DAUN SIRIH (Piper betle L.), KULIT BUAH DELIMA (Punica granatum L.), DAN RIMPANG KUNYIT (Curcuma domestica Val.) TERHADAP JAMUR CANDIDA ALBICANS. *MAKARA of Science Series*, *6*(3), 149–154. https://doi.org/10.7454/mss.v6i3.259

Tan, R. X., & Zou, W. X. (2001). Tan&Zou(2001).pdf. *Endophytes a Rich Source of Fuctional Metabolites. Nat Pro.Rep.*, (March), 448–459.

Uma Maheswari, N., & Saranya, P. (2018). Isolation and identification and phytochemical screening of endophytes from medicinal plants. *International Journal of Biology Resesarch*, *3*(1), 16–24.