

Utilization of Carbohydrates in Bamboo Shoots (*Dendrocalamus asper*) As an Alternative Media for the Growth of *Candida Albicans* Fungus

Bastian^{1*}, Firna Kamilatun Nuha², Maria Ulva³, Rossa Veronneca⁴
*e-mail: bastiandarwin51@gmail.com

^{1,2,3,4}Medical Laboratory Techology Program, Institute Of Health Sciences and Technology
Muhammadiyah Palembang, Indonesia

ABSTRACT

Candidiasis disease caused by the fungus *Candida albicans* is a health problem in Indonesia. The chronic nature of the disease gets worse if it is accompanied by a fungal infection, which usually goes undiagnosed and untreated. Culture techniques can be used to identify the fungus *Candida albicans*. Mushroom culture in the laboratory requires a medium containing nutrients such as a carbohydrate source and a nitrogen source for growth. These nutrients can be found in bamboo shoots which contain high carbohydrates, protein, fat, vitamins, and crude fiber. So that bamboo shoots can be used as raw material for alternative mushroom growth media. The purpose of this study was to utilize bamboo shoots as a medium for the growth of *Candida albicans* herbal medicine. This research included collecting bamboo shoots, making rice bran media, inoculation of *Candida albicans* fungus on bamboo shoots media, and observing fungal growth. *Candida albicans* fungal colonies were confirmed by macroscopic and microscopic observations. The results showed that the fungus *Candida albicans* grew on both media, namely Bamboo Shoots Flour Media and *Sabouraud Dextrose Agar* (SDA) media at 37°C for 48 hours. The conclusion of this study is that bamboo shoots media can be used as a medium for fungal growth. Bamboo shoots media can be used as an alternative medium for synthetic media to grow *Candida albicans*.

Keywords: Alternative Media, Bamboo Shoots, *Candida albicans*

INTRODUCTION

Indonesia is a country with a tropical climate, high humidity, so that microorganisms grow well, including mushrooms. One of the fungi that can cause infection is *Candida albicans*. This fungus can cause a disease known as *candidiasis*. *Candidiasis* is caused by the fungus *Candida* sp., which attacks the mouth, vagina, nails, skin, bronchi or lungs (Fadli, 2017).

Candida albicans infection can be tested in the laboratory *Candida albicans* infection can be tested in the laboratory. Laboratory tests that can be carried out are microscopic examination, molecular tests, serological tests, and culture systems. The culture culture system is useful for

knowing the type of fungus as well as for confirming macroscopic results. In culture culture systems, candida infection is achieved by isolating *Candida albicans* from patients on culture medium (Marchamtia Sarah Nur Awalia Fajari & Qurrohman, 2021). Growth media is a substance in which a mixture of nutrients is used for the growth and reproduction of fungi (Fadli, 2017).

Growth media according to their contents are divided into two, namely synthetic media and non-synthetic media (Prayekti, Endah; Lukiyono, 2022). Synthetic media are media whose contents are known in detail, namely the organic and inorganic materials added must be

pure, so the cost is relatively high. Synthetic media commonly used for mushroom growth are media that are rich in carbohydrates, one of which is Sabouraud Dextrose Agar (SDA) media. This media supports fungal growth because the pH is relatively low, namely pH 4.5-5.6, so that this pH can inhibit bacterial growth (Basarang & Rianto, 2018). While The non-synthetic media is media that uses natural ingredients. These materials are often unknown chemical content in detail but are often used because they are easy to prepare and relatively low cost. Microbial research is often constrained by growth media due to its high price and difficulty to obtain, especially in developing countries like Indonesia. Several studies have used natural materials as alternative media, such as (Basarang & Rianto, 2018) bamboo shoots (Nadiarta, 2017).

Bamboo shoots are part of the shoots that come from rhizomes or nodes that will emerge from the ground, bamboo shoots are a source of protein, namely in 100 grams of bamboo shoots there are 2 to 2.5 grams of protein. Bamboo shoots also contain calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na) and other minerals. (Rahmawati, 2021). The content of bamboo shoots shows the fiber content in bamboo shoots such as 30.99% hemicellulose, 37.55% cellulose, and 4.05% lignin. In addition, bamboo shoots also contain oligosaccharide components, namely sucrose and raffinose 4.55% (Silaban et al., 2017).

In fungal culture systems, testing is required for growth and identification. In general, the media that becomes the gold standard is in the form of ready-to-use media. The price of the media at a weight of 500 g reaches 3.5 million (Junaedi et al., 2022). The high price of gold standard media and the availability of natural resources in Indonesia with various

nutrients needed by microorganisms have encouraged researchers to utilize these natural resources for the growth of fungi, including bamboo shoots.

MATERIALS AND METHODS

This research uses a type of research design post test only control group. The research was carried out in the Microbiology Laboratory of the Faculty of Science and Technology, Muhammadiyah Institute of Health Sciences, Palembang, which is located at Jalan Jendral Ahmad Yani 13 Ulu, Seberang Ulu II District, Palembang City, South Sumatra 30262.

The tools used in this research were handscoon, knife, test tube, cotton, gauze, plastic tray, Erlenmeyer, blender, measuring cup, pipette, filter paper, flour sifter, analytical balance, petridisk, incubator, dry heat oven, autoclaves and microscopes. The materials used in this research were bamboo shoots, bacteriological agar, aquadest, pure culture of *Candida albicans* fungus, Sabaroud Dextrose Agar (SDA) medium, benedict, and 0.9% NaCl.

Bamboo shoots are collected from the Muara Enim area. The bamboo shoots are processed to produce flour which is used as an alternative medium for the growth of the fungus *Candida albicans*. To prove whether the bamboo shoot flour used contains sugar or not, a Benedict test is carried out on bamboo shoots (Wahyuningsih, 2019). In this test as much as 2 grams of bamboo shoot flour was added to 5 mL of Benedict's reagent and put into a test tube then homogenized and heated over spiritus and the bamboo shoots sample turned orange and then compared with the negative control.

The media for making bamboo shoot flour was carried out by weighing 5 grams of bamboo shoot flour and then adding 5 grams of bacteriological agar powder, 1 gram of glucose, and 0.040

grams of chloramphenicol and then adding 400 mL of distilled water. The medium of bamboo shoot flour is heated on a hot plate until completely dissolved. Erlenmeyer's mouth was gagged with cotton covered with sterile gauze. Then sterilized using an autoclave for 15 minutes at 121°C. After the sterilization process is complete, the media is take from the autoclave. The bamboo shoot media is allowed to cool to 45-50°C and then 15-20 mL is poured into a petri dish and allowed to harden and solidify. The media for making bamboo shoots was carried out with several concentrations, namely the concentration of bamboo shoots 100%, 80%, 50% and 25%.

Prior to the identification of *Candida albicans*, *Candida albicans* was rejuvenated with Sabouraud Dextrose Agar media because this media is a selective medium for fungi and yeast, so that it can see growth and identify *Candida albicans* (Nadziroh et al., 2018).

Microscopic observation of *Candida albicans* fungus is by germ tube test. This test was carried out using blood serum which had been incubated for 30 minutes, then the serum was mixed with *Candida* sp. and cover using a deck glass. Then incubated for 3 hours then observed and read the results. Then the data were analyzed using the One Way Anova Test with the help of the SPSS program.

RESULTS AND DISCUSSION

Candida albicans can grow on bamboo shoots because all the nutrients needed by *Candida albicans* are available in alternative media. This can be seen by the growth of *Candida albicans* fungus which forms small round colonies like yeast. This is in accordance with the statement from (Sundari et al., 2021) which states that fungi can grow and reproduce, and require a medium that

includes all the nutrients needed by the fungus.

Carbohydrate Test on Bamboo Shoots

Necessary nutrition According to (Yuniliani et al., 2018), The sugar that affects the growth of mushrooms is glucose. Glucose is a type of monosaccharide which is a source of energy and a growth medium for fungi in the metabolic system. Monosaccharides are simple sugars which are the building blocks of carbohydrates which cannot be broken down by hydrolysis in the form of vitamins, nitrogen, carbon/carbohydrates as constituents. Benedict's test serves to prove whether the substance contains glucose or not by demonstrating the presence of a brick red/orange precipitate at the bottom of the tube (Wahyuningsih, 2019).



Figure 1. Benedict's test results on bamboo shoot flour (orange in color) and negative control benedict's test (blue in color)

The bamboo shoot media contains the nutrients needed by the fungus (carbohydrates), the Benedict's test was carried out. In this test it can be said that the bamboo shoot flour contains quite high monosaccharides with the color of the bamboo shoots being orange (Wahyuningsih, 2019).

Identification of *Candida albicans* with a Germ Tube Test

The fungus *Candida albicans* is a single cell that is round to oval in shape which reproduces by forming budding

cells called blastospores. Blastospores will elongate and join together to form pseudohyphae or pseudohypae (Santri, 2017). Microscopic observation of *Candida albicans* fungus is by germ tube test. This test aims to confirm whether the growing colonies are *Candida albicans* fungi. Observations from this test are used to find Pseudohypae indicating that the fungus is *Candida albicans* (Duarsa & Dwija, 2020).

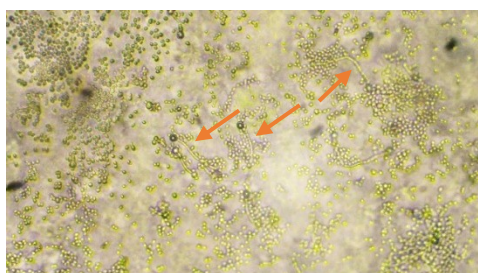


Figure 2. Germ Tube Test Results

In figure 2, *Candida albicans* is found in an oval shape like a tube extending from the yeast cell and at the end attached to the yeast cell, there is visible shrinkage (no constriction) (Pujawati et al., 2019). According to the research (Mulyati et al., 2019) it was stated that to differentiate *Candida albicans* from other types of *Candida*, a germ tube test was carried out. Because the germ tube test is positive, the species of fungus is *Candida albicans*.

Results of Total Colonies of *Candida albicans* Fungus in SDA Media and Bamboo Shoots Media Concentrations of 100%, 80%, 50% and 25%

The results of the number of *Candida albicans* fungal colonies on SDA media and bamboo shoot media at concentrations of 100%, 80%, 50% and 25% are as follows in the figure 3:

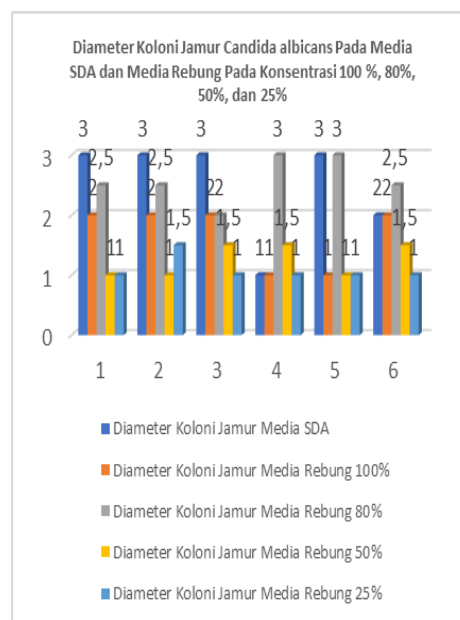


Figure 3. Bar Graph Comparison of the Number of *Candida Albicans* Fungus Colonies in SDA Media and Bamboo Shoots Concentrations of 100%, 80%, 50%, and 25%.

In the bar graph above it can be seen that the average number of colonies on SDA media is 46 Cfu/mL. The average number of *Candida albicans* colonies at a concentration of 100% was 80 Cfu/mL, at a concentration of 80% it was 47 Cfu/mL, at a concentration of 50% it was 47 Cfu/mL, and at a concentration of 25% it had an average of 48 Cfu/mL.

The graph above it can be concluded that *Candida albicans* mushroom colonies grow the most at a concentration of 100%, which is an average of 80 Cfu/mL compared to concentrations of 80%, 50% and 25%. Shoots media with a concentration of 100% had an average number of colonies more than the number of colonies growing on SDA media. But for *Candida albicans* colonies that are close to standard media is at a concentration of 80% with an average of 47 Cfu/mL.

Results Diameter of *Candida albicans* Fungus Colonies on SDA media and Bamboo Shoots Media Concentrations of 100%, 80%, 50% and 25%

The results of *Candida albicans* mushroom colony diameters on SDA media and bamboo shoot media at concentrations of 100%, 80%, 50% and 25% were as follows in the figure 4:

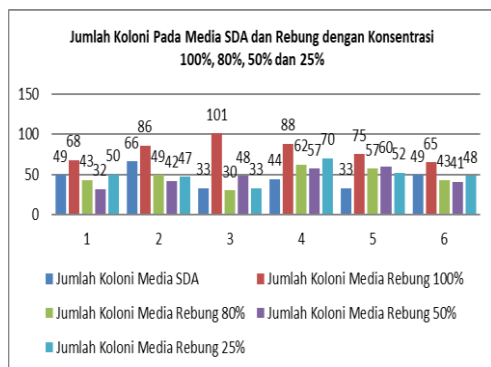


Figure 4. Bar Graph Comparison of *Candida albicans* Colony Diameters on Bamboo Shoots Media Concentrations of 100%, 80%, 50%, 25% and SDA Media.

The results of the examination of the cultivation of *Candida albicans* fungi cultures can be said to be successful if *Candida albicans* fungi colonies are found in alternative media from this bamboo shoot material (Tamam, 2019). In the bar graph above it can be seen that the colony diameter on SDA media has an average of 2.5 mm. In bamboo shoot media with a concentration of 100%, the average diameter of the mushroom colony was 2 mm. In bamboo shoots media with a concentration of 80%, the average colony diameter was 3 mm. In bamboo shoot media with a concentration of 50%, the average colony diameter was 1 mm and at a concentration of 25%, the average colony diameter was 1 mm.

The explanation above it can be concluded that bamboo shoots with a concentration of 80% have the highest average compared to bamboo shoots with a concentration of 100%, 50% and 25%. Also, bamboo shoots with a concentration

of 80% had an average colony diameter higher than the average diameter of mushroom colonies growing on SDA media, which was 3 mm. But for mushroom colonies *Candida albicans* which is close to standard media is at a concentration of 80% with an average diameter of 3 mm.

Hypothesis Test Results (Statistics Test)

Based data analysis the diameter and growth of colonies on SDA media and bamboo shoots media with concentrations of 100%, 80%, 50%, and 25% from repeated ANOVA test results on differences in the number of colonies and colony diameters on SDA media, bamboo shoot alternative media (*Dendrocalamus asper*) can be concluded that the hypothesis is accepted because a significant value of $p < \alpha$ ($\alpha = 0, 05$). Statistically, there was a difference in the number of *Candida albicans* colonies between the SDA media and bamboo shoot (*Dendrocalamus asper*) alternative media.

This research was made with concentrations of 100%, 80%, 50%, and 25%. Some of these concentrations were made with the aim of seeing the comparison of the number of fungal colonies at each concentration (Danela et al., 2019). Media control treatment (Sabroaud Dextrose Agar media) was carried out to see the impact of contaminants on the media used and fungal control treatment (media and mushrooms) to see the initial number of fungal colonies *Candida albicans* (Octaviani & Fadila, 2018).

The growth of fungi on the media is due to the presence of sufficient carbon and protein sources in the bamboo shoots media. Nutrients in the form of chemical elements or compounds from the environment are used by cells as chemical components of cells. Mushrooms will grow well in media rich in carbohydrates and nitrogen (Basarang & Rianto, 2018).

Bamboo shoot flour media uses bamboo shoots as a source of nutrition for mushrooms because they contain carbohydrates (2-2.5%) and protein (1.6-2.5%) (Septadiya & Syadi, Yunan Kholifatuddin Isworo, 2018). Carbohydrates and their derivatives are the main substrate for carbon metabolism, fungi are also able to degrade proteins from their environment and use them as a source of nitrogen and carbon (Naim et al., 2020). The nutrients in the growth medium must contain all the elements necessary for the biosynthesis of the new organism (Basarang & Rianto, 2018).

The growth of *Candida albicans* colonies growing on bamboo shoots media has the same characteristics as *Candida albicans* colonies growing on SDA media, the difference is only in the number and diameter of *Candida albicans* colonies. There are differences in the number and diameter of colonies in SDA media and bamboo shoot media because the nutritional content of bamboo shoots and SDA media is different (Sundari et al., 2021).

CONCLUSION

Based on the results it can be concluded that bamboo shoots can be used as a raw material for making *Candida albicans* growth media with an optimum concentration of 80% because at this concentration the number of diameters and the number of colonies approaches standard media (SDA media). *Candida albicans* can grow on bamboo shoots because all the nutrients needed by *Candida albicans* are available in alternative media. This can be seen by the growth of *Candida albicans* fungus which forms small round colonies like yeast.

Acknowledgments

Thank you to the Ministry of Education and Culture of the Republic of Indonesia and the Institute of Health Sciences and Technology for providing both material and formal support in this Student Creativity Program. We also thank the Head of the Medical Laboratory Technology Study Program IKesT Muhammadiyah Palembang, Head of the IKesT Muhammadiyah Palembang Student Affairs Unit for the permits and support that has been given in the Student Creativity Program activities that have been carried out.

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