GROWTH OF CANDIDA ALBICANS ON MODIFIED MEDIA BASED ON COOWBEANS (VIGNA UNGUICULATA L. WALP)

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Abstract. One fungus that can cause infection is Candida albicans. Supporting examinations to confirm the diagnosis can be carried out by growing the fungus in fungal growth media. The abundance of natural resources can be used as substitute raw materials for growth media for microorganisms. Cowpea is a natural ingredient that contains quite high nutrients, is widely available, easy to cultivate, has a fast harvest time, and is relatively affordable. This research aimed to determine the growth and morphology of Candida albicans on modified cowpea media. The method used was laboratory experimental and five variations were made to differ in the amount of cowpea added, namely 5 g (F1), 10 g (F2), 15 g (F3), 20 g (F4), 25 g (F5). The research showed that Candida albicans fungus grew on the modified cowpea media. The morphology of Candida albicans colonies in all cowpea-modified media formulations is characterized by smooth round colonies, yellowish white with a characteristic yeast odor. Candida albicans fungi in F1, F2 and F3, produce blastospores. Candida albicans fungi in F4 and F5 produce blastospores, pseudohyphae, and chlamydospores. Cowpea-modified media can be used as an alternative growth medium for Candida albicans, the best seen from its morphology is F5 with the addition of 25 g of cowpea.
Keywords: *modified media, cowpea, Candida albicans, fungus*

Introduction

Fungi are one of the causes of infection, especially in tropical countries (Delost, 2018). One of the fungi that can cause infection is Candida albicans (Jawetz et al., 1991). The disease caused by Candida sp is known as candidiasis (Rollenhagen et al., 2020). Candidiasis often attacks the skin, oral mucous membranes, and respiratory tract (Talapko et al., 2021; Zebua et al., 2021). Supporting examinations to confirm the diagnosis can be carried out by growing mushrooms on fungal growth media (Nurdin, 2021). Growth media are used for laboratory purposes such as isolation to support the diagnosis of a disease and to reproduce stock cultures (Atmanto et al., 2022). Currently, various types of instant media are available, including media for fungal growth, such as SDA (Sabouraud Dextrose Agar). SDA media is the best medium for fungal growth because it contains glucose which is a source of nutrition for fungal growth (Wasilah et al., 2023). However, there are several obstacles to the use of instant media, including that instant media is ready-to-use factory production, relatively expensive, hygroscopic, and difficult to obtain (Ningrum et al., 2018). To overcome this condition, it is necessary to look for alternative media with basic materials that are more affordable and abundantly available.

The abundance of natural resources can be used as substitute raw materials for growth media for microorganisms. This is because these materials are easy to obtain do not require expensive costs, and have almost the same content as factory-produced media (Prayitno, 2017). Several studies have been successfully carried out using nutrients from natural ingredients to create alternative media for mushroom growth (Rahmayanti et al., 2022; Sasongkowati, et al., 2022; Jamilatun et al., 2020). Cowpea is a natural ingredient that contains quite high nutrients, is widely available, easy to cultivate, has a fast harvest time, and is relatively affordable (Trustinah and Mejaya, 2017). Based on this description, research was carried out on the growth of Candida albicans on modified cowpea (Vigna unguiculata L.Walp) media. This research aims to determine the growth and morphology of Candida albicans on modified cowpea media.

Materials and Methods

This research uses laboratory experimental methods. Tools used include Petri dishes (*pyrex*), watch glass (*pyrex*), analytical balance (*labex*), beaker (*pyrex*), measuring cup (*pyrex*), stir bar, hotplate, bunsen burner, tube needle, spatula, test tube (*pyrex*), erlenmeyer (*pyrex*), incubator (*vienna*), autoclave (hirayama autoclave hve-50), oven (memmert). The materials used include *Candida albicans* isolates, SDA media (*Sabouraud Dextrose Agar*), cowpeas, sucrose, agar-agar, distilled water, LPCB (*Lactophenol Cotton Blue*), chloramphenicol antibiotics, wrapping paper. Research procedures include sterilizing equipment, making media, inoculating *Candida albicans* and observing the growth and identification of *Candida albicans*.

Sterilization of tools: Heat-resistant tools are sterilized using an oven at 180^{o}C for 120 minutes. Meanwhile, tools that are not heat resistant are sterilized using an autoclave at 121^{o}C for 15 minutes (Jamilatun and Lukito, 2024; Jamilatun and Safitri, 2023).

Making SDA Media: SDA media was weighed at 19.5 g, and put into an Erlenmeyer, then 30 mg of the antibiotic chloramphenicol was added and dissolved in 300 mL of distilled water. The media is heated and homogenized with a magnetic stirrer on a hotplate. The media was sterilized by autoclaving at 121^{o}C for 15 minutes. Next, the media was poured into a petri dish aseptically and left to solidify (Jamilatun, 2023).

Making modified Cowpea media: The cowpeas are weighed according to the amount in the formulation, then ground by blending without adding water, and then filtered using gauze until the cowpea filtrate is obtained. The filtrate was put into an Erlenmeyer and 5 g of sugar was added, 20 g of agar, 100 mg of the antibiotic chloramphenicol, and distilled water was added until a final volume of 1000 mL was obtained. The media is heated and homogenized using a stirrer on a hotplate. The media was then sterilized by autoclaving at 121^{o}C for 15 minutes. The sterilized media is poured into Petri dishes aseptically and left to solidify (Jamilatun et al., 2020; Nuryati and Sujono, 2017) (*Table 1*).

Table 1. *Cowpea Modified Media Formulation.*

Material	F1	F2	F3	F4	F5
Cowpeas (g)	5	10	15	20	25
Agar-agar (g)	20	20	20	20	20
Sucrose (g)	5	5	5	5	5
Chloramphenicol (mg)	100	100	100	100	100

Aquades (mL)	1000	1000	1000	1000	1000

Note: Modified media formula with the addition of cowpeas F1 (5 g), F2 (10 g), F3 (15 g), F4 (20 g), F5 (25 g).

Growth of Candida albicans: *Candida albicans* fungus was inoculated on modified cowpea media and SDA media. Petri dishes that have been planted with *Candida albicans* are wrapped in wrapping paper and placed in the incubator. Samples were incubated at 37^{o}C for 48 hours (Sasongkowati et al., 2022).

Identification of Candida albicans: Observations were made macroscopically and microscopically. Macroscopic observations can be seen directly by observing the color and shape of the colony (Jamilatun, 2022). Microscopic observations were carried out using staining. Fungal staining was carried out by taking 1 loop of the *Candida albicans* fungus colony from the media, then attaching it to the surface of a glass object and dripping it with LPCB dye, covering it with a covered glass, and observing it with a microscope.

Results and Discussion

The results of the growth of the *Candida albicans* fungus on modified cowpea media and SDA media are presented in *Table 1*. This research was conducted to determine the growth and morphology of *Candida albicans* on modified cowpea media. Cowpea-modified media is a natural seed medium whose composition consists of cowpeas as a source of carbohydrates. Sucrose is a substitute for dextrose and agar is a solidifier. Media preparation is carried out aseptically. The addition of the antibiotic chloramphenicol aims to prevent the growth of bacteria in the media. The media that has been made is then poured into a sterile petri dish and left to solidify. There were 5 variations of the modified media used in the research with different amounts of cowpea added, namely 5 g (F1), 10 g (F2), 15 g (F3), 20 g (F4), 25 g (F5). Meanwhile, SDA media was used as a control. The growth media that has been made is then inoculated with the Candida albicans fungus using the streak method (Jamilatun et al., 2020). Then incubate and observe. The growth of Candida albicans colonies on modified cowpea media and SDA media gave the same colony picture (*Figure 1*). The observation results can be seen in the following image (*Table 2*).

***Table 2**. Growth of Candida albicans on Modified Cowpea Media and SDA media.*

Formulas	Growth of *Candida albicans*
F1	There is growth
F2	There is growth
F3	There is growth
F4	There is growth
F5	There is growth
SDA	There is growth

Note: Modified media formula with the addition of cowpeas F1 (5 g), F2 (10 g), F3 (15 g), F4 (20 g), F5 (25 g), SDA (Control).

Figure 1. *Morphology of Candida albicans on SDA media. Macroscopic (left), smooth round colonies, yellowish white with a characteristic yeast odor. Microscopically (right), there are blastospores, chlamydospores and pseudohyphae.*

The growth of *Candia albicans* colonies found in all cowpea-modified media formulations is characterized by smooth round colonies, yellowish white with a characteristic yeast odor. The Candida albicans fungus in F1 (*Figure 2*) and F2 (*Figure 3*) produces blastospores that appear very small and do not contain pseudohyphae. The *Candida albicans* fungus in F3 (*Figure 4*) produces larger blastospores but there are no pseudohyphae yet. The *Candida albicans* fungus in F4 (*Figure 5*) and F5 (*Figure 6*), produces larger blastospores, and there are pseudohyphae and chlamydospores. These results show that the higher the concentration of cowpea added to the modified media, the better the growth of *Candida albicans.* This is by the statement that one of the growth parameters is the increase in cell volume, due to the addition of protoplasm and nucleic acid compounds which involve DNA synthesis and mitotic division (Wasilah et al., 2023). The results of this study are by previous research which stated that cowpea flour can be used as an alternative medium for the growth of microorganisms (Salsabila et al., 2023; Nuryati and Sujono, 2017).

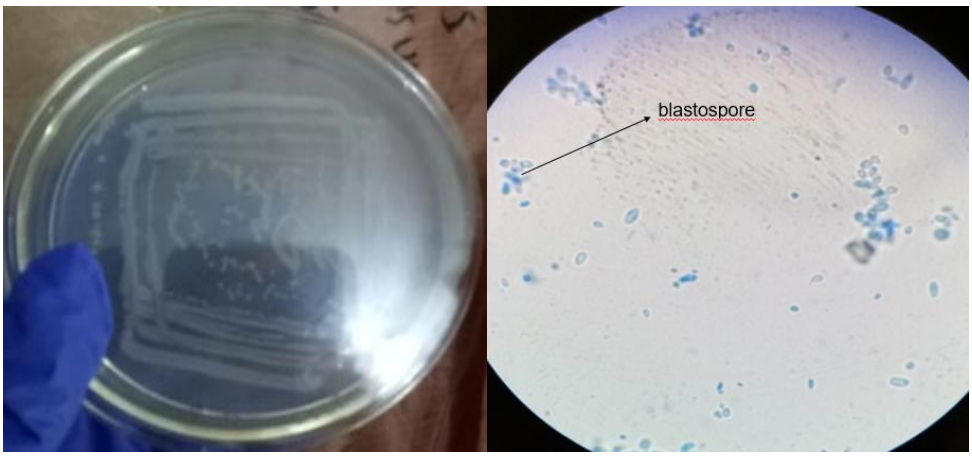

Figure 2. *Morphology of Candida albicans on F1 modified media. Macroscopic (left), smooth round colonies, yellowish white with a characteristic yeast odor. Microscopically (right), there are blastospores.*

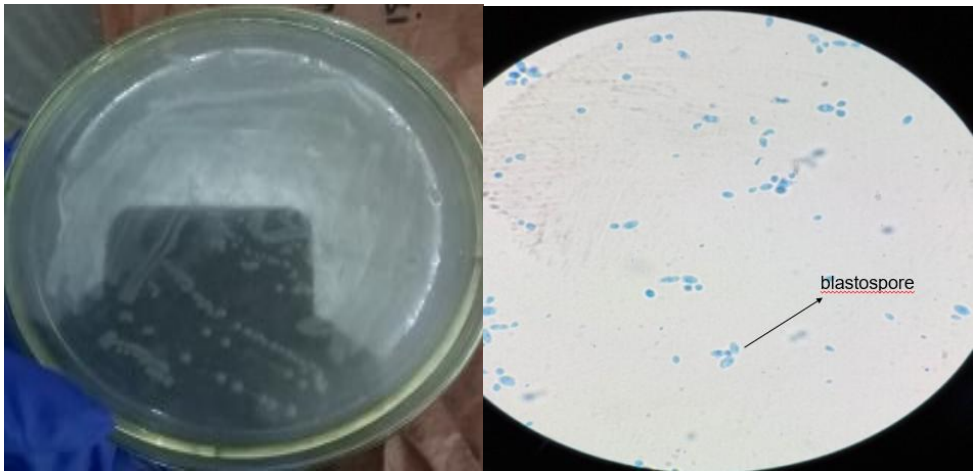

Figure 3. *Morphology of Candida albicans on F2 modified media. Macroscopic (left), smooth round colonies, yellowish white with a characteristic yeast odor. Microscopically (right), there are blastospores.*

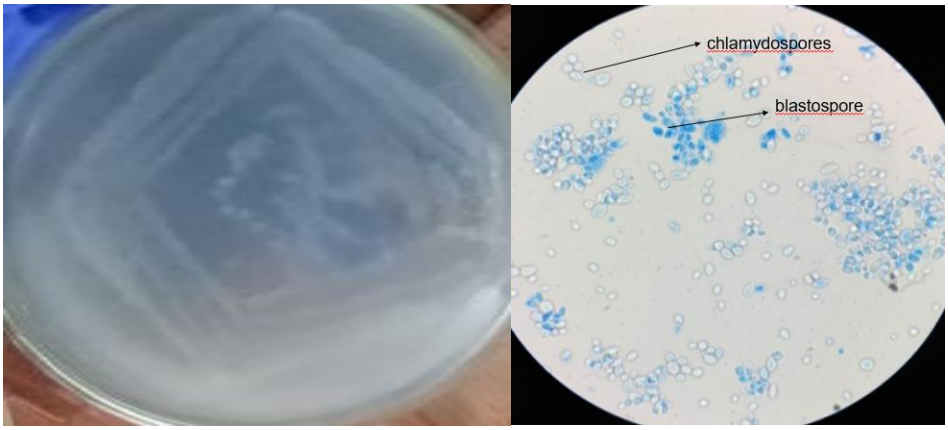

Figure 4. *Morphology of Candida albicans on F3 modified media. Macroscopic (left), smooth round colonies, yellowish white with a characteristic yeast odor. Microscopically (right), there are blastospores and chlamydospores.*

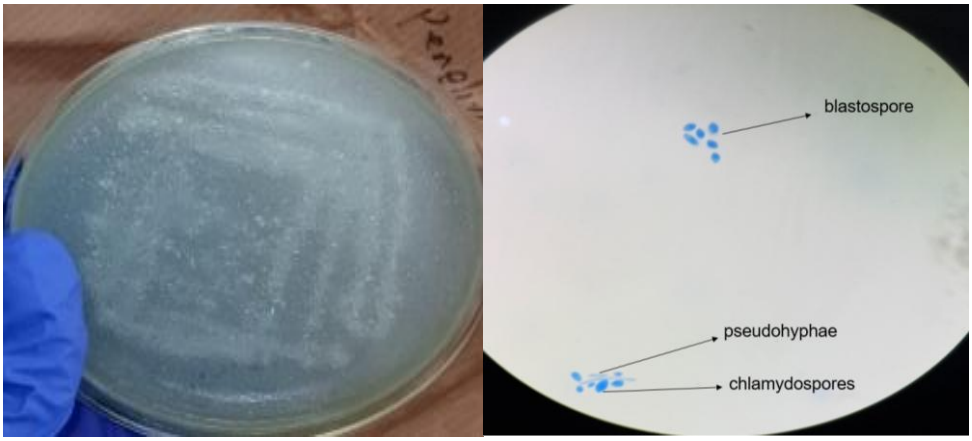

Figure 5. *Morphology of Candida albicans on F4 modified media. Macroscopic (left), smooth round colonies, yellowish white with a characteristic yeast odor. Microscopically (right), there are blastospores, chlamydospores, and pseudohyphae.*

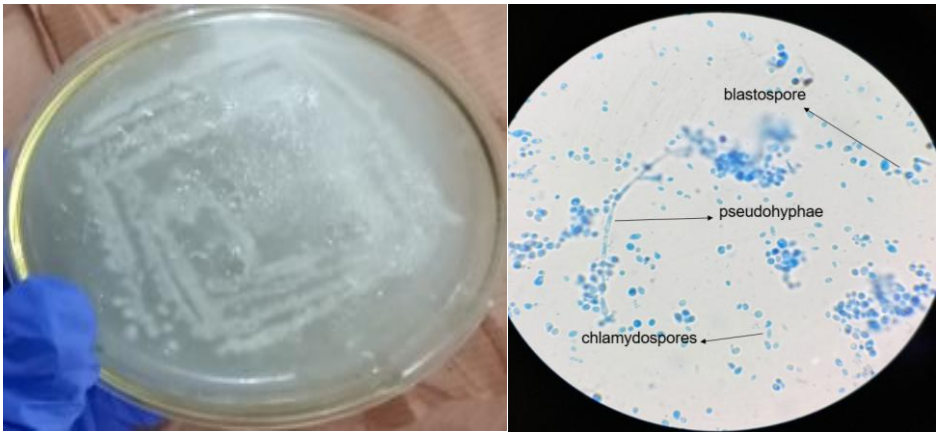

Figure 6. *Morphology of Candida albicans on F5 modified media. Macroscopic (left), smooth round colonies, yellowish white with a characteristic yeast odor. Microscopically (right), there are blastospores, chlamydospores and pseudohyphae.*

Fungal growth is influenced by several factors, including the substrate. Substrate is the main source of nutrients for fungi (Wasilah et al., 2023). Microorganisms in their growth require nutrients which are a source of energy. The nutritional content of cowpeas, which is very complex and rich in nutrients, can influence the growth of the *Candida albicans* fungus. Cowpea flour has a protein content of 26.42%, carbohydrates of 63.04%, and fat of 1.44% (Efendi et al., 2023). The nutritional content in cowpeas can be used as a source of nutrition for the growth of microorganisms in alternative media. Carbohydrates are the main substrate for carbon metabolism. Apart from that, fungi are also known to have the ability to break down proteins in their environment and use them as a source of nitrogen. Carbohydrates also act as an energy source in the formation of fungal cells (Efendi et al., 2023; Nuryati and Sujono, 2017). The complex nutritional content of cowpeas is utilized by fungi to grow and develop, as well as the *Candida albicans* fungus which requires carbohydrates to grow and develop (Rahmayanti et al., 2022). With the modified medium, cowpeas can be used as an alternative medium for fungal growth, especially *Candida albicans*. This modified media is more cost-effective than instant media, it is simple to make and the materials are easy to obtain.

Conclusion

Candida albicans can grow well on modified cowpea (Vigna unguiculata L. Walp) media. The morphology of *Candida albicans* in modified cowpea media is not different from the morphology in SDA media so modified cowpea media can be used as an alternative growth medium for *Candida albicans*. The best-modified medium, seen from its morphology, is F5 with the addition of 25 g of cowpea.

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Conflict of interest

The authors confirm that there is no conflict of interest involve with any parties in this research study.

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