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EFFECT OF CASSAVA TUBER PROCESSING ON *Candida albicans* GROWTH ON MANIHOT DEXTROSE AGAR

Popi Patilaya[1]*, Imam Bagus Sumantri[2], Jane Melita Keliat[3]
[1]*Pharmaceutical and Food Analysis Programme, Faculty of Vocational, Universitas Sumatera Utara. Jalan Bioteknologi No.2, Kampus USU, Medan, 20155, Indonesia.*
[2]*Faculty of Pharmacy, Universitas Sumatera Utara. Jalan Tridharma No.5, Kampus USU, Medan, 20155, Indonesia*
*Corresponding Author: popi.patilaya@usu.ac.id

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ABSTRACT

This study evaluated the effect of cassava tuber processing on *Candida albicans* growth on Manihot Dextrose Agar (MDA). The sliced cassava tubers were divided into three groups. G1 was milled and dried in an oven at 60°C. G2 was directly dried in the oven. G3 was milled without drying. Each group was soaked in distilled water (1,000 mL) for 15 min and filtered with a flannel cloth. The filtrate was oven-dried, then pulverized to obtain the cassava powder. The powder (8 g) was combined with dextrose (20 g) and agar (15 g). The mixture was suspended in distilled water (1,000 mL), boiled, and sterilized at 121°C for 15 min. The solution was placed into a petri dish and allowed to form agar media. The media derived from G1, G2, and G3 were considered as F1, F2, and F3. A suspension of *C. albicans* was cultured on the media and incubated for 48 h. The fungal growth was observed by calculating the viable colonies. The data were analyzed with one-way ANOVA and followed by Duncan's test at the confidence level of 95%. The results showed that *C. albicans* grown on media F1, F2, and F3 had colony numbers of 128±2.08 x 10^5 cfu/mL, 64±5.57 x 10^5 cfu/mL, and 44±3.51 x 10^5 cfu/mL, respectively. Statistical analysis indicated a significant difference in the fungal growth on the three media ($p < 0.05$). This study proved that the processing methods of cassava tubers for MDA significantly affected the growth of *C. albicans*.
Keyword: *Candida albicans*, Fungi, Media, Cassava

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi efek pengolahan umbi singkong terhadap pertumbuhan *Candida albicans* dalam Manihot Dextrose Agar (MDA). Umbi singkong yang telah dirajang dibagi menjadi tiga kelompok (G1, G2, dan G3). G1 dihaluskan dan dikeringkan dalam oven pada 60^{O}C. G2 langsung dikeringkan dalam oven. G3 dihaluskan tanpa dikeringkan. Tiap kelompok di rendam dalam 1.000 mL air suling, didihkan selama 15 menit, dan disaring dengan kain flanel. Filtrat kemudian dikeringkan dalam oven, lalu dihaluskan menjadi serbuk. Sebanyak 8 g serbuk umbi singkong dicampur dengan 20 g dekstrosa dan 15 g agar. Campuran disuspensikan ke dalam 1.000 mL air suling, dididihkan dan disterilkan pada 121^{o}C selama 15 menit. Larutan dipindahkan ke dalam cawan petri dan dibiarkan membentuk media agar. Media yang diperoleh dari G1, G2, dan G3 masing-masing disebut sebagai F1, F2, dan F3. Suspensi *C. albicans* dibiakkan dalam media tersebut dan diinkubasi selama 48 jam. Pertumbuhan jamur diamati dengan menghitung koloni yang tumbuh. Data dianalisis dengan ANAVA satu arah dan dilanjutkan dengan uji Duncan pada taraf kepercayaan 95%. Hasil penelitian menunjukkan bahwa *C. albicans* tumbuh dalam media F1, F2, dan F3 dengan jumlah masing-masing 128±2,08 x 10^5 koloni/mL, 64±5,57 x 10^5 koloni/mL, dan 44±3,51 x 10^5 koloni/mL. Hasil analisis statistika menunjukkan perbedaan yang nyata pertumbuhan jamur pada ketiga media ($p < 0,05$). Penelitian ini membuktikan bahwa metode pengolahan umbi singkong untuk media MDA mempengaruhi pertumbuhan *C. albicans*.
Keyword: *Candida albicans,* Jamur, Media, Singkong

1. Introduction

Cassava tubers contain carbohydrates, proteins, fats, and fibers [1–3]. The cassava tubers also consist of minerals such as sodium, calcium, potassium, magnesium, iron, zinc, copper, and manganese [4]. The presence of those chemical contents leads cassava tubers as a potential source for a medium of fungal growth, including *Candida albicans*. Fungi are capable of using different carbon sources for the synthesis of carbohydrates, lipids, nucleic acids, and proteins. Sugars, alcohols, proteins, lipids, and polysaccharides are oxidized to produce energy for fungi. Fungi also require nitrogen sources for the synthesis of amino acids, nucleic acids, proteins, chitin, and various vitamins. The fungi use nitrogen in the form of nitrate, nitrite, ammonium, or organic nitrogen [5].

C. albicans is commonly cultured in a fresh or dried cassava tuber-based medium. The cassava tubers are soaked in distilled water to obtain the cassava infusion. Fungal medium is a combination of the cassava infusion with other ingredients [6]. However, the cassava infusion should be made fresh for each formula. The medium can also be formulated by mixing water-insoluble dried powders [7,8]. This method is less effective because the water-insoluble materials may interfere with the observation of the fungal growth [9]. In addition, the cassava tubers also contain cyanide compounds. The cyanides are volatile and found in free form or bound to glucosides and cyanohydrin. These compounds are toxic to fungi, so they may affect the growth of *C. albicans* [10].

The previous study reported that dried extract of cassava tuber infusion can be used as a medium for *C. albicans* growth. The best formula contains dried cassava tuber extract, sugar, and agar. However, the raw materials used are still fresh tubers without a process of cyanide removal. In addition, the cassava extract powder was obtained by drying with a water bath, so it's time-consuming [11]. According to Rashmi and Devatha (2021), the water removal process can be accelerated through drying in an oven [12]. Therefore, this work was conducted to observe the effect of cassava tuber processing of Manihot Dextrose Agar (MDA) on *C. albicans* growth.

2. Methods

2.1. Chemicals

Chemicals used in this study include dextrose (Himedia), agar powder (Himedia), potassium dihydrogen phosphate (Merck), dicalcium hydrogen phosphate (Merck), concentrated sulphuric acid (Merck), barium chloride (Merck), potato dextrose agar (Himedia), soybean casein digest agar (Himedia), soybean casein digest broth (Himedia), Sabouraud dextrose agar (Himedia), sodium chloride (Merck), and distilled water (Rudang Jaya).

2.2. Preparation of Plant Materials

Cassava tubers were collected at Tambak Rejo Village, Pancur Batu District, North Sumatra Province (Indonesia) in August 2023. The cassava tubers were separated from the peel. The cassava flesh was washed, drained, and sliced with a thickness of 2-5 mm. The sliced materials were divided into three groups. Group 1 was milled and dried in an oven at 60°C to obtain the powder materials. Group 2 was dried in the oven at 60°C without milling. Group 3 was milled without a drying process.

2.3. Preparation of Media

Media preparation was carried out by adopting the procedure of Patilaya et al. (2015) [11]. Each group of the plant materials (100 g) was soaked in 1,000 mL of distilled water and boiled for 15 min. The suspension was filtered with a flannel cloth. The filtrates were dried in an oven at 60°C and pulverized to get the cassava powder. Media were formulated by mixing the cassava powder (8 g) with dextrose (20 g) and agar powder (15 g). The media formulated with Group 1, Group 2, and Group 3 of cassava powder were considered F1, F2, and F3, respectively. The mixture was suspended in 1,000 mL of distilled water and boiled until completely dissolved. The media were sterilized by autoclaving for 15 minutes at 121°C. Potato dextrose agar (PDA) was used as a comparison medium. The powder of PDA was suspended in 1,000 mL of distilled water, boiled, and autoclaved at 121°C for 15 minutes. After sterilization, the media were placed in a petri dish and allowed to solidify at room temperature. The flowchart for MDA preparation is shown in Figure 1.

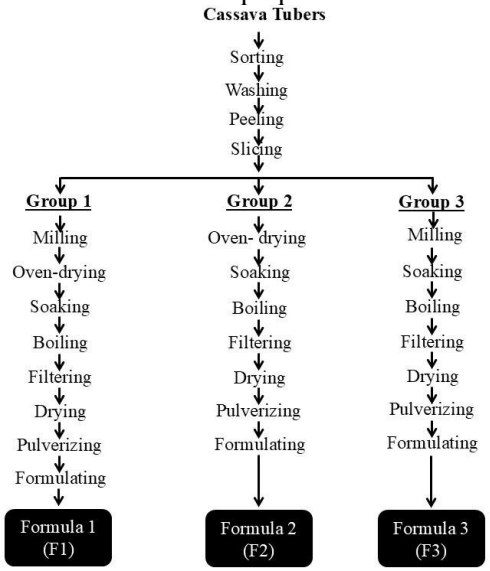

Figure 1. Process flowchart for the preparation of MDA

2.4. Observation of *C. albican*s Growth

Colonies of *C. albicans* were inoculated on potato dextrose agar media and then incubated at 28°C for 48 h. Fungal colonies were suspended in 10 ml of 0.9% NaCl and vortexed until it homogenized. The suspension was adjusted with McFarland standard No. 0.5 to provide a turbidity of 108 cfu/mL. The fungal suspension (1 mL) was transferred into 9 mL of 0.9% NaCl solution and diluted to 1:10,000. The inoculum was cultured on the test medium (F1, F2, F3, and FDA) and incubated at 28°C for 48 h. The growth of *C. albicans* was observed by calculating the number of viable colonies. The experiment was performed in triplicate. Data was presented as means ± standard error [10].

2.5. Data Analysis

The viable colonies of *C. albicans* on the tested media were analyzed statistically using IBM SPSS Statistics 22.0 software. The data was analyzed with one-way ANOVA and Duncan's test at the confidence level of 95%.

3. Results and Discussion

This study was concerned with the processing effects of cassava tubers used as a fungal medium. The tuber processing involved powdering-oven drying, slicing-oven drying, and grinding without drying. We observed the growth of C. albicans on Manihot Dextrose Agar (MDA) media to assess the processing effects. *C. albicans* is a common human fungal pathogen that causes invasive candidiasis. Hence, media for fungal identification is essential [13]. Although commercial media, such as CHROM Agar, Sabouraud Dextrose Agar, and Potato Dextrose Agar (PDA), are available, they are expensive [14].

The results indicate that *C. albicans* is a single-celled fungal species. A similar result was also reported by Putri et al. (2022) [15]. Therefore, one can observe fungal growth by calculating the number of colonies on the agar plate [16]. As presented in Figure 2, the results showed that *C. albicans* grew well on MDA (F1, F2, and F3), as well as on PDA. The number of colonies of *C. albicans* on F1 was 128±1.20 × 10^5 cfu/mL. The colonies of *C. albicans* were also detected on F2 and F3 with the total number of 64±3.21 x 10^5 cfu/mL and 44±2.48 x 10^5 cfu/mL, respectively. Interestingly, the results indicated that the fungal colonies on F1 were higher than on F2 and F3, respectively. However, the growth *of C. albicans* on PDA, with a colony count of 146±2.33 x 10^5 cfu/mL, was higher than on F1, F2, and F3 media. Those results were confirmed by statistical analysis (Table 1-3). There was a significant difference in *C. albicans* growth on PDA, F1, F2, and F3 ($p < 0.05$).

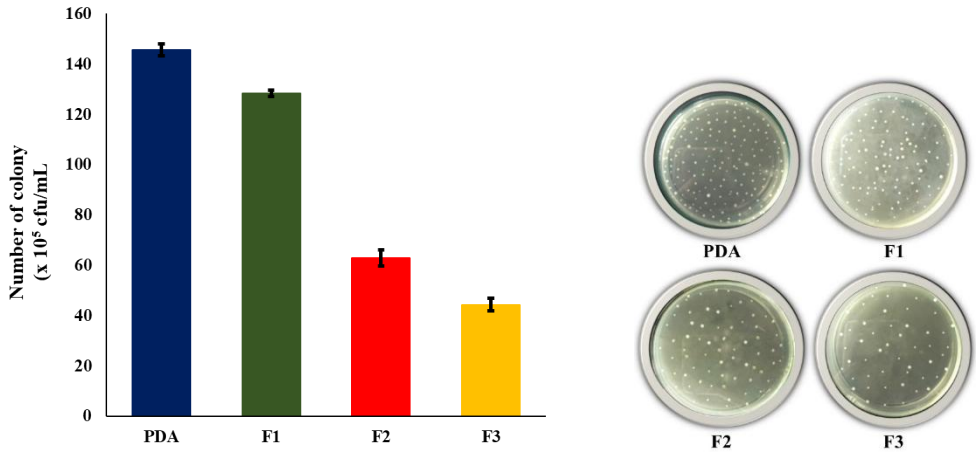

Figure 2. Growth of *C. albicans* on Manihot Dextrose Agar (MDA) with different formulas (F1, F2, F3) and Potato Dextrose Agar (PDA) after incubation at 28°C for 48 hours.

This study showed that the cassava tubers processed by milling-oven drying produced the best medium for *C. albicans* growth compared to the cassava tubers derived by slicing-oven drying and milling without drying methods (Figure 2). This phenomenon may be due to the difference in nutrient content in those media. Agbara and Babagana (2022) reported the significant effects of drying on the nutritional composition of cassava tubers. After oven-drying, the carbohydrate content of cassava tubers slightly increased to 3.87%. The mineral levels in cassava tubers, such as K, Ca, Mg, Fe, and Zn, also increased by over 40% after drying processing. However, the protein and lipid levels of cassava tubers decreased to 8.37% and 33.06%, respectively [17].

Table 1. Homogeneity test of variances of *C. albicans* colony growth on MDA and PDA.

Levene Statistic	df1	df2	Sig.
0.833	3	8	0.513

Table 2. One-way ANOVA of *C. albicans* colony growth on MDA and PDA.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21806.667	3	7268.889	454.306	0.000
Within Groups	128.000	8	16.000		
Total	21934.667	11			

Table 3. Duncan's post hoc tests of *C. albicans* colony growth on MDA and PDA.

Types of Media	N	Subset for alpha = 0.05			
		1	2	3	4
F3	3	44.3333			
F2	3		63.0000		
F1	3			128.3333	
PDA	3				145.6667
Sig.		1.000	1.000	1.000	1.000

The cassava tubers also contain cyanogenic compounds, such as linamarin, lotaustralin, and free HCN. Linamarin and lotaustralin are enzymatically degraded to form free cyanide [18]. About 98.16 mg of cyanide is present in 100 mg of cassava tuber [19]. The presence of cyanide in the medium can inhibit the respiration of *C. albicans* and decrease ATP production [20]. This phenomenon is the reason that tubers should be processed before being used for fungal media. Okonkwo et al. (2019) reported that cyanide content in cassava tuber decreased to 89.64% after drying with an oven at 50°C [21]. Another study indicated that oven-drying treatment decreased 48.15% of the cyanide level in the cassava tubers [22]. Decreasing the size of cassava tubers also reduces the cyanide content. Cyanide levels decreased to 86.76% when the tubers were processed into powder [23]. Meanwhile, slicing cassava tubers reduced cyanide levels by up to 50% [24]. According to Ndubuisi et al. (2018), the most effective method for removing cyanide is drying the small pieces or powder of cassava tubers [25].

Viji et al. (2018) also proved the effects of cassava tuber processing on fungal growth. The fungal growth on the media-based infusion cassava tuber was higher than on the media-based cassava powder [26]. Another study revealed *C. albicans* grown in media-based cassava tuber infusion with a colony number of 862 cfu/mL [27]. However, Safitri and Qurrohman (2022) reported the fungal growth on the same medium with 58.7×10^{11} cfu/mL in the number of colonies [28]. Geographical differences and the variety of cassava tubers used may influence the variation in results [29]. Of course, those variables will affect the nutritional composition of the resulting media.

The present study has successfully identified the effects of cassava tuber processing on the growth of *Candida albicans* on Manihot Dextrose Agar. The best method for cassava tuber processing is milling-oven drying. However, the correlation between the cassava tuber processing and the nutritional composition and cyanide remains unclear. Therefore, more research is required to elucidate this issue.

4. Conclusion

The results indicated that the processing of cassava tubers with Manihot Dextrose Agar significantly affected *C. albicans* growth. The best fungal growth occurs on media-based cassava tubers processed by milling-oven drying, compared to those processed by slicing-oven drying and milling without any drying methods. However, more research is required to elucidate the relationship between the processing of cassava tubers and their nutritional composition.

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6. Conflict of Interest

Authors declare that there is no conflict of interest with any party regarding this study. We also declare that this study does not require ethical approval.

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