Comparison of effectiveness of red beans (Phaseolus vulgaris L.) and candlenut (Aleurites moluccana (L.) Willd) as a replacement for media sabouraud dextrose agar for Candida albicans growth

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Abstract. Fungi require nutrients, a source of energy and environmental conditions for their growth. Sabouraud Dextrose Agar is a general medium frequently for fungal growth. The high cost and the difficulty of multiplying SDA media have encouraged researchers to find alternative media from red beans and candlenuts that contain carbohydrates and protein which are as a source of nutrition for the growth of Candida albicans. The research aimed determine the growth of Candida albicans on red beans (Phaseolus vulgaris L.) and candlenuts (Aeurites moluccana (L.) Willd ) media and to determine the effectiveness of both media. The method of the research is experiment (completely randomized design) with 3 treatments and 9 repetitions. The average of colonies on red beans media of 181.7 CFU / ml, and candlenuts media of 113.8 CFU / ml with an average colony diameter red beans media of 1.19 mm and 1.12 mm candlenut media. Observations processed statistically using One Way Annaova. The result showed a significant difference between the count and diameter of Candida albicans growth colonies on red beans (Phaseolus vulgaris L.) and candlenut (Aeurites moluccana (L.) Willd) media, followed by the Tukey test with the best media results being red beans (Phaseolus vulgaris L.) media.

1. Introduction
Candida albicans is a normal flora of the oral cavity, digestive tract and urinary tract. Candida albicans change into a pathogen if the body's stomach is decreases. The Candida albicans yeast is diagnosed by clinical examination. To strengthen the diagnosis, culture and microscopic examination is necessary so that the type of fungals that causes it can be determined. Candida albicans cultures generally used isolation techniques on growth media.

The medium used as a growth medium for Candida albicans in the laboratory is SDA (Sabouraud Dextrose Agar). SDA contains various nutrients for fungal growth such as carbohydrates and protein. Usually the composition of SDA is arranged the basic needs of fungus because they live in natural habitats[1]. The preparation of Candida albicans growth media in the laboratory generally uses media in the form of a formula and is commercially available. SDA media are marketed in a ready-to-use state at a high price and difficult to obtain.

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To resolve the issue, it is necessary to modify the media to speed up the diagnosis of fungal. Unfortunately, synthetic modification is very complicated, requires precision, takes a long time and is a chemical that is expensive. Therefore, it is necessary to make alternative media as a substitute for fungal growth media, including using red beans and candlenuts which contain protein and carbohydrate sources that allow fungal growth.

In previous research, red beans (Phaseolus vulgaris L.) was tested as an alternative growth medium for Tricophyton sp. The results showed that SDA media can be used as an alternative medium to substitute SDA because the diameter of colony in red beans media is almost the same as SDA media [2]. Red beans and candlenut have never been used as an alternative medium to substitute Sabouraud Dextrose Agar for the growth of Candida albicans. Therefore, this study was conducted to see the comparison of effectiveness.

Based on the description above, researchers have conducted research on the comparison of the effectiveness of red beans (Phaseolus vulgaris L.) and candlenut (Aleurites moluccana (L.) Willd) as an alternative medium to replace sabouraud dextrose agar for the growth of Candida albicans.

2. Material and Methods

2.1. Material

The materials used were red beans, candlenut, sabouraud dextrose agar media, universal pH indicator, chloramphenicol antibiotics, aquadest, Physiological Nacl and flour agar. Candida albicans strains were obtained from the Padang Health Laboratory.

2.2. Methods

This study used an experimental method by count of the number and measuring diameter of colony Candida albicans on sabouraud dextrose agar and alternative media of red beans (Phaseolus vulgaris L.) and candlenut (Aleurites moluccana (L.) Willd) with 9 repetitions.

2.3 Techniques for making red beans(Phaseolus vulgaris L.) alternative media

Pounded Red beans with a mortal until crushed. then filtered to separate coarse powder. 25 grams of fine powder then added with 250 ml of aquadest and homogenized in Erlenmeyer. Add 5 grams of red beans solution to agar flour and 1 gram of dextrose and then homogenize it on the hotplate. Close the Erlenmeyer with a lid made of cotton wrapped in newspaper and tied with corn rope. sterilize the media using an autoclave at 121°C for 15 minutes. Put The chloramfenicol antibiotic into the solution after autoclaving around 45-50°C. Measured the pH of the media used a universal indicator. After that the media is poured into the Petri disk and wait until it hard and ready to use.

2.4 Techniques for making candlenut alternative (Aleurites moluccana (L.) Willd) media

Pounded candlenut with a mortal until crushed. then filtered to separate coarse powder. 25 grams of fine powder then added with 250 ml of aquadest and homogenized in Erlenmeyer. Add 5 grams of candlenut solution to agar flour and 1 gram of dextrose and then homogenize it on the hotplate. Close the Erlenmeyer with a lid made of cotton wrapped in newspaper and tied with corn rope. sterilize the media using an autoclave at 121°C for 15 minutes. Put The chloramphenicol antibiotic into the solution after autoclaving around 45-50°C. Measured the pH of the media used a universal indicator. After that the media is poured into the Petri disk and wait until it hard and ready to use.

2.5 Inoculation technique of Candida albicans on alternative media of red beans (Phaseolus vulgaris L.), Candlenut (Aleurites moluccana (L.) Willd) and sabouraud dextrose agar controlMedia

Candida albicans isolates were first made into a suspension with the addition of physiological NaCl then adjusted to the Barium Sulfate Standard. Candida albicans suspension was smeared on alternative media of red beans (Phaseolus vulgaris L.), candlenut (Aleurites moluccana (L.) Willd) and on SDA control media, used an ose round. Incubated at 37°C for 3 days and all work was carried out aseptically.
2.6 Macroscopic and microscopic examination
Macroscopic examination of fungal growth on alternative media of red beans (Phaseolus vulgaris L.), and candlenuts (Aleurites moluccana (L.) Willd) in a petri dish was assessed included the count of colonies and colony size. Microscopic examination of fungal colonies grew on alternative media of red beans and candlenuts used gram stain and observed its pseudohyphae.

2.7 Statistic analysis
The data obtained were analyzed statistically used the one way anova test and tukey test with an error rate of 5% used the IBM SPSS Statistics program 24.

3. Results and Discussion
The results showed that Candida albicans can grew well on alternative media of red beans and candlenut after incubation of 24 hours. Counts of the number and diameter of Candida albicans colonies were carried out for 9 repetitions (Table 1). The count of colonies in the alternative medium of red beans showed an average of 181.7 colonies with an average diameter of colony was 1.19 mm, while the average count of colonies on candlenut media was 113.8 with an average diameter of colony was 1.12 mm, where in SDA control media the mean count of colonies was 215.5 and the average diameter of colony was 1.88 mm (Figure 1, 2). then, the data obtained were processed statistically used one way anova test to identify the effectiveness of alternative media to the growth of Candida albicans. Based on the results of the analysis of the Anova showed a significant value (p <0.05), this indicates that there is a significant difference between alternative media and can influence the growth of Candida albicans. The results of anova test followed up by tukey test to find out the differences in the growth effectiveness of candida albicans colonies on alternative media. Tukey test results showed that the red bean medium was more effective in replaced SDA media as a growth medium for Candida albicans.

Table 1. Growth results of Candida albicans after 2x24 hours incubation

| Repetition | Count of Colony CFU/ml (10^3) | Diameter of colony (mm) |
|------------|-------------------------------|------------------------|
|            | SDA (control) | Read beans Media | Candlenut Media | SDA (control) | Read beans Media | Candlenut Media |
| 1          | 186           | 204              | 102             | 2.10          | 1.75             | 1.30          |
| 2          | 250           | 166              | 140             | 2.25          | 1.05             | 1.10          |
| 3          | 170           | 190              | 100             | 2.52          | 1.70             | 1.40          |
| 4          | 265           | 169              | 118             | 1.55          | 1.10             | 1.77          |
| 5          | 198           | 198              | 95              | 2.27          | 1.00             | 0.92          |
| 6          | 210           | 216              | 133             | 1.62          | 1.45             | 0.87          |
| 7          | 212           | 140              | 89              | 1.57          | 0.40             | 0.80          |
| 8          | 250           | 164              | 135             | 1.70          | 1.20             | 1.02          |
| 9          | 198           | 188              | 112             | 1.42          | 1.10             | 0.90          |
| Mean       | 215.4         | 181.7            | 113.8           | 1.88          | 1.19             | 1.12          |
Figure 1. Comparison of the average count of *Candida albicans* colonies on alternative media for red beans (*Phaseolus vulgaris* L.), candlenuts (*Aleurites moluccana* (L.) Willd) and SDA control media.

Figure 2. Comparison of the average diameter of *Candida albicans* colonies on alternative media for red beans (*Phaseolus vulgaris* L.), candlenuts (*Aleurites moluccana* (L.) Willd) and SDA control media.

Macroscopically, *Candida albicans* colonies that grew on alternative media of red bean (*Phaseolus vulgaris* L.) and candlenut (*Aleurites moluccana* (L.) Willd) showed the growth of colonies that were round, cream colored, flat edges and 1-2 mm in diameter. On agar medium, within 48 hours the temperature of 37°C *Candida* produced creamy and yeast-smelling colonies. Growth can be seen from the increase in colonies in the growth medium of about 1-3 mm [3]. Determination of the best media must consider two things, namely the count of colonies and colony size [4]. The macroscopic form of *Candida albicans* can be seen in (Figur 3,4)
Figure 3. Growth of *Candida albicans* on SDA control media

**Figure 4.** Growth of *Candida albicans* on alternative media A: Read beans (*Phaseolus vulgaris* L.) Media B: Candlenut (*Aleurites moluccana* (L.) Willd) media

Microscopically, the end cells were oval-shaped, germinated and purple in color. This is consistent with the characteristics of *Candida albicans* growth small oval, thin-walled, sprouting, measuring 3-6 μm and gram positive. The microscopic form of *Candida albicans* can be seen in (Figur 5).

**Figure 5.** Microscopic *Candida albicans* used gram stain magnification of 10x40

The count of colonies on the *sabouraud dextrose agar* control medium in order to be more so, the colony diameter size was bigger than the red beans (*Phaseolus vulgaris* L.) and candlenut (*Aleurites moluccana* (L.) Willd) media. SDA usually used culture medium because of its simple formulation and its ability to support growth in various fungus. SDA media has a composition consisting of dextrose as a source of carbon, sugar and protein as a source of energy for growth[1]. SDA has a high glucose content for fungus growth. Glucose is a type of monosaccharide which is a source of energy and a medium for fungus growth in the metabolic system[5].

The factors that can affect the growth of fungal colonies are nutrient content and fiber content[4]. *Candida albicans* really needs nutrients in the form of nutrients such as protein, carbohydrates, crude
fiber for growth. Carbohydrates provide a greater carbon content which acts as a building block. In addition, media components also play a role in supporting fungus growth. Because good fungal growth showed that it not only contains the right nutrition but also contains nutrients in the right proportion \[^6\]

Alternative media from red beans is the best alternative media because it has the count of colonies and colony diameters that are almost comparable to the colonies of SDA control media. The showed that the composition of nutrients, especially carbohydrates in the media, is in accordance with the nutritional needs of *Candida albicans*. The alternative media for red beans (*Phaseolus vulgaris* L.) contains 56.2 grams/% carbohydrates and 22.1 g/% protein \[^6\]. The nutritional content of red beans in 100 grams contains 336 kcal of energy, 22.30 g protein, 1.5 g fat and 61.20 g carbohydrates \[^7\].

*Candida albicans* yeast growth in alternative media of candlenut (*Aleurites moluccana* (L.) Willd) is good compared to red beans because candlenut only contains 8 grams of carbohydrates, 63 grams of fat. The high fat content in candlenut can be an inhibit factor for fungal growth because fat can affect cell surface tension and cell membrane permeability, and *Candida albicans* yeast do not have enzymes that can hydrolyze fat so that nutrients were difficult to absorb into cells. Nutritional content of candlenut contains 55-65% oil, 55% saturated fatty acids and 94.2% unsaturated fatty acids. the fatty acids contained in candlenut (*Aleurites moluccana* (L.) Willd) can inhibit *Candida albicans*, because the fatty acids act as an antifungal which can kill *Candida albicans* \[^8\].

4. Conclusion
Based on the research, can be concluded that the alternative media for red bean and candlenut (*Aleurites moluccana* (L.) Willd) can be used as an alternative medium for the growth of *Candida albicans*. Alternative media from red beans is the best alternative media because it has the count of colonies and diameters of colony that are almost comparable to the colony of SDA control media.

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