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Research Report

Inhibition of 10% *Alpinia galanga* and *Alpinia purpurata* rhizome extract on *Candida albicans* growth

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ABSTRACT

Background: *One of normal oral flora that found in human oral cavity is Candida albicans (C. albicans). The overgrowth of this species can lead to opportunistic infection known as candidiasis. Two natural plants, Alpinia galanga rhizome and Alpinia purpurata rhizome, are natural remedies containing flavonoid, saponin, tannin, and triterpenoid used as antifungal component.* ***Purpose:*** *This experimental laboratory study is aimed to determine the inhibition of Alpinia galanga rhizome and Apinia purpurata rhizome on the growth of C. albicans.* ***Methods:*** *Alpinia galanga rhizome and Alpinia purpurata rhizome were extracted in ethanol solvent using soxhletation method. The ratio test was conducted on those two extracts at the concentration of 10% toward the growth of C. albicans through agar diffusion method.* ***Results:*** *The results showed that 10% Alpinia galanga rhizome extract and 10% Alpinia purpurata rhizome extract were able to inhibit the growth of C. albicans, about 7.33 mm for Alpinia galanga rhizome extract and 6 mm for Alpinia purpurata rhizome extract. The results of statistical tests using independent samples t-test showed that there was no significant difference between the inhibition of 10% Alpinia galanga rhizome extract and that of 10% Alpinia purpurata rhizome extract.* ***Conclusion:*** *In conclusion 10% Alpinia galanga rhizome extract and 10% Alpinia purpurata rhizome extract have weak inhibition on C. albicans growth.*

Key words: *Candida albicans, candidiasis, Alpinia galanga rhizome, Alpinia purpurata rhizome*

ABSTRAK

Latar belakang: *Candida albicans (C. albicans) merupakan flora normal yang terdapat dalam rongga mulut, jika keseimbangannya terganggu maka jamur tersebut akan menjadi patogen dan dapat menyebabkan infeksi dalam rongga mulut yaitu kandidiasis. Lengkuas rimpang putih maupun lengkuas rimpang merah merupakan tanaman yang mengandung senyawa antijamur berupa flavonoid, saponin, tanin, dan triterpenoid.* ***Tujuan:*** *Penelitian eksperimental laboratoris ini dilakukan untuk mengetahui perbandingan daya hambat ekstrak lengkuas rimpang putih (Alpinia galanga) dengan ekstrak lengkuas rimpang merah (Alpinia purpurata) terhadap pertumbuhan C. albicans.* ***Metode:*** *Ekstrak lengkuas rimpang putih maupun lengkuas rimpang merah diperoleh dengan metode soxhletasi. Dilakukan pengujian perbandingan kedua ekstrak pada konsentrasi 10% terhadap pertumbuhan C. albicans dengan menggunakan metode difusi agar.* ***Hasil:*** *Ekstrak lengkuas rimpang putih 10% dan ekstrak rimpang lengkuas merah 10% mampu menghambat pertumbuhan C. albicans dengan daya hambat rata 7,33 mm untuk ekstrak lengkuas rimpang putih dan 6 mm untuk ekstrak lengkuas rimpang merah 10%. Hasil uji statistik menggunakan independent sampel t test menunjukkan tidak ada perbedaan bermakna antara respon hambat ekstrak lengkuas rimpang putih dan ekstrak lengkuas rimpang merah.* ***Kesimpulan:*** *Dari hasil penelitian ini dapat disimpulkan bahwa ekstrak lengkuas rimpang putih 10% dan ekstrak lengkuas rimpang merah 10% memiliki daya hambat yang lemah terhadap pertumbuhan C. albicans.*

Kata kunci: *Candida albicans, kandidiasis, lengkuas rimpang putih, lengkuas rimpang merah*

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INTRODUCTION

Unlike bacteria which considered as prokaryotic microorganisms, fungi are considered as eukaryotic microorganisms. One of the most commonly found fungi in oral cavity is *Candida*. *Candida* is a normal fungus found in oral cavity, gastrointestinal tract, genital tract, and sometimes in skin.[1] *Candida* is also known to present approximately in 40-60% of human population.[1,2] There are actually 200 different species of *Candida*, including *Candida albicans (C. albicans), Candida glabrata (C. glabrata), Candida crusei (C. crusei), Candida tropicalis (C. tropicalis),* but *C. albicans* in oral cavity is the most common species that may cause disease. As much as 75-90% of fungal infections in humans are triggered by *C. albicans*.[1-3] Another report also shows that 40-60% of the oral cavity in healthy adult population contains *C. albicans* with small concentration (200-500 cells/ml saliva).[3,4]

C. albicans, as a normal flora in oral cavity will become pathogenic and cause candidiasis when a person has risk factors of the excessive growth of *C. albicans*. Those risk factors are triggered not only by local factors such as xerostomia, but also by the use of topical corticosteroids, prostheses, and smoking habit. In addition, there are also systemic factors considered as risk factors, such as either the use of antibiotics and systemic corticosteroids or hormonal changes caused by pregnancy or diabetes mellitus. Candidiasis is often found in people infected with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) triggered by weak immunity.[2,3]

Nowadays a lot of medicine can be used to overcome infections caused by *Candida*. Generally, infections caused by *Candida* can be solved by the following three groups of agents, namely Polyenes, Azole and DNA analogues. Those agents can be used based on the type and severity of infections.[1] However, these drugs tend to be expensive and have more side effects in long-term use, such as being resistant to fungus and being harmful when used in patients with hepatitis (Azole class).[2] Therefore, it is necessary to look for alternative drugs that is cheap, relatively safe to use, and easy to get, such as Alpinia.

Alpinia is herbal medicine known as anti-fungal derived from Zingeberaceace family. Ginger rhizome is traditionally used as a drug to treat skin diseases, especially those caused by fungi.[5] There are two kinds of galanga, namely red ginger (*Alpinia purpurata* rhizome) and white ginger (*Alpinia galanga* rhizome). White ginger is widely used as spices or herbs, while red ginger is widely used as herbs.[5,6]

White ginger, furthermore, contains 1% greenish yellow volatile oil and some other compounds. One of the researchs on exposed white ginger shows that some other compounds were succesfully isolated from white ginger, such as acetoxychavicol acetate considered as antifungal.[7,8] Another compound that also has antifungal activity is a diterpene isolated from white ginger. Further studies also show that diterpene works by altering the lipid membrane of *C. albicans* then resulting in the changes of the permeability of its membrane.[9] A test conducted on *Alpinia galanga* rhizome extract with concentration of 10% even shows that there were antifungal activities against *C. albicans* in vaginalis candidiasis.[8] On the other hand, red ginger (*Alpinia purpurata*) contains flavonoids, saponins and tannins. One of the functions of flavonoids is as antimicrobial and antifungal activities.[10] For those reasons, this study is aimed not only to determine the inhibition response of white ginger extract (*Alpinia galanga*) and red ginger extract (*Alpinia purpurata*) toward the growth of *C. albicans,* but also to determine the ratio of the inhibition response of 10% white ginger extract (*Alpinia galanga*) and 10% red ginger extract (*Alpinia purpurata*) to the growth of *C. albicans*.

MATERIALS AND METHODS

This study is considered as an experimental laboratory study conducted at Biological Chemistry Laboratory of Mathematic and Natural Science Faculty and at Microbiology Laboratory of Veterinary Faculty, University of Syiah Kuala, in Banda Aceh. The unit of analysis in this study was the dosage form of *C. albicans* ATCC 10231 obtained from Microbiology Laboratory of Medical Faculty, University of Indonesia. Samples used in this study were white ginger (*Alpinia galanga*) and red ginger (*Alpinia purpurata*) obtained from Peunayong Market in Banda Aceh.

At the first stage, *C. albicans* was obtained by using a sterile loop, and then was grown in sabouraud dextose agar (SDA) media. Next, it was incubated in incubator at 37 ˚C for 24 hours until its growth occured. Afterwards, *C. albicans* grown on SDA media was identified by Gram by using staining method, and then was observed under a microscope with a magnification 1000. The preparation of suspension was conducted to produce *C. albicans* more. This process was conducted by inoculating *C. albicans* into 10 ml peptone, and then compared with the turbidity level of Mc. Farland solution about 0.5 (1.5 x 106 CFU/ml).

At the next stage, white ginger (*Alpinia galanga*) and red ginger (*Alpinia purpurata*) obtained was extracted. Fresh white ginger (*Alpinia galanga*) and red ginger (*Alpinia purpurata*) obtained as much as 1 kg were washed, cut crosswise, and dried at 60 ˚C for 15 minutes before they were then mashed. Next, the powder of white ginger (*Alpinia galanga*) and red ginger (*Alpinia purpurata*) obtained was wrapped in filter paper, and then soaked separately in 600 ml of 96% ethanol in soxhletasi tool until the solvent droplets were colorless. Afterwards, the filtrate mix with the solvent was evaporated with rotary evaporator at 40 °C to obtain pure extract. Since the amount of the extracts obtained was not sufficient, then the gingers

Table 1. The classification of the inhibition response[12]

Diameter of light zone	Inhibition response toward the growth
20–30 mm	+++ (Strong)
11–20 mm	++ (Moderate)
6–10 mm	+ (Weak)
0	–

were needed to be added.[10, 11] The pure extracts obtained was then diluted by using sterile aquadest water to obtain specific concentration about 10%.

The effectiveness of those extracts of 10% white ginger (*Alpinia galanga*) and red 10% ginger (*Alpinia purpurata*) obtained toward the growth of *C. albicans* was tested by using agar diffusion method with sterile SDA media. Afterwards, the suspension of *C. albicans* that had been measured was poured about 0.1 ml by using Eppendorf pipette into the media surface, and then was smoothed by using a sterile spreader bar (Hocky sterile). The next step is to put disc papers, each of which had been soaked in white ginger extract (*Alpinia galanga*) 10% and red ginger extract (*Alpinia purpurata*) 10%, while disc papers soaked in aquadest were used as negative control. Those three media were then incubated at 37 °C for 24 hours. The zone of inhibition toward the growth of fungi could be observed by measuring and recording the diameter of inhibition zone using long slide in millimeters. The observation was conducted three times. The parameters that would be observed was the diameter of inhibition zone of *C. albicans* growth. The results obtained way interpreted using Table 1.[12]

Data obtained were tested with Shapiro-Wilk test and were normally distributed, later analyzed with parametric Independent Sample t-test with 95% confidence level. The test showed a significant result ($p<0.05$).

RESULTS

C. albicans that had been cultured on SDA media for 48 hours and stored in the incubator had a spherical colony shape with slightly convex, creamy colour, and soft surface as well as yeast aroma. Confirmatory test was conducted using Gram staining test. The results on *C. albicans* using Gram staining test microscope observation with 1000 magnification showed that there were round cells, pseudohyphae, and purple smooth tube hyphae indicating that the strain was *C. albicans* (Figure 1).

Test of the inhibition response was conducted on *Alpinia galanga* rhizome extract and *Alpinia purpurata* rhizome extract using agar diffusion method and disc papers soaked in each of those solution extracts. The test was repeated three times.

Statistical test used was Independent Sample t-test. There was no significant differences between *Alpinia galanga* rhizome and *Alpinia purpurata* rhizome in inhibiting the growth of *C. albicans* with $p = 0.057$.

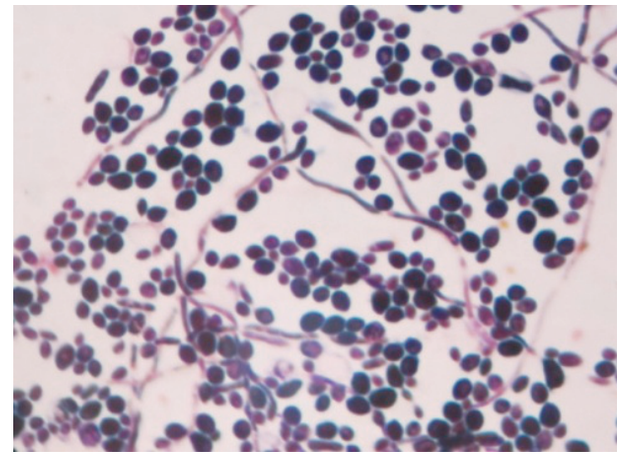

Figure 1. The result of Gram staining test**.** a) Cell, b) Pseudohyphae, c) Hyphae.

DISCUSSION

The results of *C. albicans* cultured on SDA media showed that colonies formed were slightly convex, creamy, and soft surface as well as yeast aroma smell. The morphology was is similar to the statement of Jawetz,[13] Kayser[15] and Rippon[14]. SDA is considered as a standard medium for culturing *C. albicans* since it contains dextrose and peptone to support the growth of *C. albicans*, however, several other fungi can also grow on this medium because SDA is not a selective medium only for *C. albicans* therefore a confirmation test is required to ensure the process of culturing is not contaminated with other fungi.[16] *C. albicans* have a thick structure as composed of chitin, mannan, and glucan which causes stiffness and low permeability so that when moistened with alcohol, the cell walls cannot be penetrated and the violet gentian dye is still remained in the cell and will make cells looks purple.[14]

The 10% *Alpinia galanga* rhizome extract and 10% *Alpinia purpurata* rhizome extract were capable of inhibiting the growth of *C. albicans* with the average of the inhibition response about 7.33 mm for *Alpinia Galanga* rhizome extract and 6 mm for *Alpinia purpurata* rhizome

Tabel 2. The Independent Sample t-test results diameter of the inhibition zone of the growth of *C. albicans* of the extracts of *Alpina galangal* rhizome and *Alpina purpurata* rhizome (mm)

Extract	Mean of inhibition diameter	p
10% *Alpinia galanga rhizome*	7.33	0.057
10% *Alpinia purpurata rhizome*	6	

extract. Both of *Alpinia galanga* rhizome and *Alpinia purpurata* rhizome are actually two plants derived from the Family of Zingeberacea known as one of the herbs that have known benefits as antifungal.[5,10,17] *Alpinia galanga* rhizome extract and *Alpinia purpurata* rhizome extract could inhibit the growth of *C. albicans*.[8-10]

The inhibition response of *Alpinia galanga* rhizome extract toward the growth of *C. albicans* can happen because the extract contains both diterpene compounds, such as acetoxychavicol acetate, and flavonoid compounds, such as kaempferol, kaempferide, galangin, alpinin and essential oils which are antifungal compounds.[6,7] The diterpene compounds work by altering the lipid membrane of *C. albicans,* so the changes of their membrane permeability occur.[9] Meanwhile, flavonoids work by inhibiting the synthesis of nucleic acids of fungi and destabilizing the cell membranes due to the change of the nature of the fungal cell membrane that may cause the exchange of fluid in the cell. On the other hand, the inhibition response of *Alpinia purpurata* rhizome is caused by the fact that it contains saponins, tannins, flavonoids, essential oils and diterpen compounds.[18,19] Saponin works as an antifungal agent by interfering with the permeability of the fungal cell wall. The antifungal activity of saponin, however, is related to the composition of aglycone and the structure of monosaccharide unit in their sugar chain group.[20,21]

The results of the study conducted by Silvina,[8] also showed that among 30 samples of SDA containing 10% *Alpinia galanga* rhizome extract in candidiasis vaginalis patients there was no *C. albicans* grown. However, the result is different from the result in this study due to the different test method conducted. Solid dilution method was used to test the effectiveness of 10% galanga extracts, thus, it made the extract distributed, and make the contact with the fungus more effective.[8, 21] Unlike ~~that~~ previous study, in this study agar diffusion method was chosen since the process is relatively simpler, more practical and thorough. Nevertheless, this method still has weaknesses one of which causes the limitation of the average size of the inhibition zone formed. Other disadvantages of this method include the results of the testing of several samples with different antimicrobial potency cannot always be compared among them because each sample has different physical properties, such as solubility, volatility, and diffusion characteristics. Thus, the content of the extract which has good diffusion coefficient, but weak antifungal activity can diffuse into the agar well. Meanwhile, although the content of the extract has good antifungal activity, but without having good diffusion coefficient, it will affect the inhibition zones formed. In addition, another weakness of this method will also arise when comparing the inhibition zone formed from different samples. In the disc paper method, the inhibition zone will also be affected by concentrations given in the disc paper.[22]

Alpinia purpurata rhizome extract is more effective than *Alpinia galanga* rhizome extract in inhibiting the growth of *Streptococcus mutans*.[23] Similarly, it is also known that *Alpinia purpurata* rhizome extract is more effective in inhibiting *Trichopyton ajelloi* than *Alpinia galanga* one.[24] Unlike those studies, in this study it is known that there was no significant difference between *Alpinia galanga* rhizome extract and *Alpinia purpurata* rhizome extract. This is due to the differences of the structures of both of fungi.[25–28]

Based on the classification of the inhibition response according to Morales,[12] it is also known that the inhibition response of *Alpinia galanga* rhizome extract 10% and *Alpinia purpurata* rhizome extract 10% toward the growth of *C. albicans* is in the weak category (+). The cause of the weak inhibitition zone was affected by the quality of the rhizome extract obtained for test material. The quality of simplicia and natural materials that will be used as drugs can be standardized based on the method of making simplicia issued by Ministry of Health of the Republic of Indonesia. The poor quality of extract is caused by the amount of the active substances contained in the rhizome.[29] The active substances is actually affected by temperature and humidity. Alpinia have different levels of maturity. The different levels of maturity can lead to the differences of the texture and color of substances costituted.[30]

The weak inhibition response, can also be caused by the extraction method using soxhletasi method. This method has some disadvantages, the solvent used must be volatile and can only be used for the extraction of heat-resistant compounds. This negative side then may affect the amount and quality of antifungal compounds contained in the extracts of both *Alpinia galanga* rhizome and *Alpinia purpurata* rhizome contained will be damaged at high temperature. If the extracts are broken, it will affect the ability of these substances to inhibit the growth of *C. albicans*. One of the effects is that volatile oil, such as sineol is unstable and sensitive to high temperature. During the drying and withering process, plant cell membrane gradually breaks, as a result, water penetrates freely from one cell to another to form a volatile compound and the amount of water in plant cells will diffuse into the top surface.[31] The minimal inhibitory zone in 10% *Alpinia galanga* rhizome extract and 10% *Alpinia purpurata* rhizome extract may occur due to the heating process at soxhletasi period.

C. albicans grow in an environment that has a specific concentration and is still able to survive from minimum concentartion to maximum. *C. albicans* can optimally grow from at pH 4.5 to at pH 6.5.[32] Thus, the small inhibition zone of both of 10% *Alpinia galanga* rhizome extract and 10% *Alpinia purpurata* rhizome extract may be caused by the value of those extract pH that ranged in pH optimum for the growth of *C. albicans*. Similarly, according to a study conducted by Wahyuni,[24] it is also known that based on the measurement of the acidity of both of n-hexane extract and *Alpinia galanga* rhizome extract, the pH obtained was ranging from 5% to 50% that was equal to 5. This result indicates that the pH of *Alpinia galanga* rhizome extract can become a factor triggering the growth of *C. albicans* around disc papers since they are in the range of pH for

their optimum growth. It can finally be concluded that both 10% *Alpinia galanga* rhizome extracts and 10% *Alpinia purpurata* rhizome extract have weak inhibition response toward the growth of *C. albicans*.

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