EFFICACY OF BAJAKAH TAMPALA ETHANOL EXTRACT, A TYPICAL PLANT OF KALIMANTAN ISLAND (BORNEO), AGAINST CANDIDA ALBICANS BIOFILM

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Abstract: The emerging resistance to anti-fungal agents encourage the exploration of new and effective natural product showing anti-fungal activity to significantly impact the and the treatment management of biofilm associated fungal infections. In nature, most of microbes live in form of biofilms, and up to 80% of microbial infections on human body are biofilms associated. This research aimed at finding out antibiofilm activity of Bajakah tampala extract against Candida albicans. The inhibition and eradication activities toward C. albicans biofilm were tested using microtiter broth method by measuring the minimum value of biofilm inhibition concentration ($MBIC_{50}$) and minimum biofilm value eradication concentration ($MBEC_{50}$). The C. albicans biofilm structure in front of and absence of the extracts was analysed using SEM. At concentration of 1% w/v Bajakah tampala extract showed activity against C. albicans mid-phase biofilm formation and maturation phase (77.00% ± 0.01, 70.31% ± 0.01) and breakdown the established biofilms as much as 65.21% ± 0.01. The biofilm architecture analysis by SEM provided evidence that Bajakah tampala ethanol extract disturbed the extracellular polymeric substance (EPS) matrix of the C. albicans biofilm. The result obtained clearly indicate that Bajakah tampala exthanol extract can developed as a new antibiofilm candidate for the treatment of C. albicans biofilm infection.

Keywords: Bajakah tampala, biofilm, C. albicans, infection, antibiofilm

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INTRODUCTION

Candida albicans is one of the most pathogenic biofilm-forming fungi. In immunocompromised individuals, C. albicans has become an opportunistic pathogen, and one of the leading causes of morbidity and mortality worldwide . These fungi invade epithelial tissue, cause superficial infections, and are life-threatening [1].In the United States, 75% of women during their reproductive years have Candida vulvavaginitis infection, i.e., between 40-50% of patients have recurrent infections and 5-8% of patients have chronic candidiasis [2]. Of the 345 C. albicans infection cases studied in hospitals in Spain, the mortality rate was 51% due to C. albicans infection (Almirante et al., 2005). Meanwhile, in Germany, the number of deaths due to necrosectomy caused by Candida reached as much as 62% [3].

Biofilms are groups various microbial cells that bind irreversibly to the surface , enclosed in a extracellular polymer subtances (EPS) matrix, and express phenotypic changes such as growth rates and plankton cell gene changes transcription or free radicals [4]. According to data from the US National Institutes of Health (NIH), more than 60% of microbial infections are associated with biofilms [5], and up to 80% human infections are biofilm associated [6]. Biofilms as part of the microbial defense system to quite difficult of destroy for antibiotics, as a consequence pathogenic bacteria in biofilms can cause serious problems for human health [7].

Indonesia is an archipelagic country with a rich diversity of flora [8]. This wealth must be appreciated as a national asset to increase the nation's resilience and sovereignty. One of the islands in Indonesia that has varieties of plants is the island of Kalimantan (Borneo). Kalimantan has long been known as a home to a wide varieties of medicinal plants and is one of Indonesia's largest sources of plant diversity. One of the famous native plants of Borneo is Bajakah Tampala (Spatholobus littoralis Hassk) (Saputera and Ayuchecaria, 2018) [10]. However, Bajakah in the language of the Dayak tribe in Kalimantan means roots, referred to hundreds of species of climbing-twisted rooted plants in the Kalimantan rain forest [11]. Based on a preliminary qualitative test conducted by Anshari (2012), Bajakah Tampala (S. littoralis Hassk) contains phenolic compounds, flavonoids, and tannins. This is reinforced by research conducted by Saputera and Ayuchecaria

(2018), where Bajakah Tampala has been shown to have the ability to accelerate wound healing. Furthermore, other studies have shown that Bajakah Tampala has antibacterial activity [12]. Research on Bajakah Tampala on C. albicans biofilms has not been reported. Therefore, this study will explore the bioactivity of the ethanol extract of Bajakah Tampala as an antibiofilm of C. albicans .

MATERIAL AND METHODS

The gear used are Laminar Airflow, incubator (IF-2B) (Sakura, Japan), pipette micropipette (Gilson, France), multichannel micropipette (Socorex, Switzerland), polystyrene nicely ninety six flatbed microplate (Iwaki, Japan), microtiter plate reader (Optical device Ivymen 2100 -C, Spain), spectrophotometry (UV Genesys 10 experiment, 335903) (Thermo scientific Spectronic, america of the united states), autoclave (Sakura, Japan), analytical stability (AB204 -five, Switzerland). The fabric used on this have a observe turn out to be the ethanol extract of the Bajakah Tampala collected from the Forests in East Kalimantan, the same old isolate of C. albicans for biofilm formation (C. albicans ATCC 10231) from the collection of the Microbiology laboratory of the college of Pharmacy UGM, Fluconazole 1% w/v, DMSO 1% w/v, NaCl, McFarland widespread zero.five, Sterile Distilled Water, Sabouraud Dextrose Broth (SDB) Media, RPMI Media, PBS (Phosphate Buffer Saline) solution, 1% w / v Quartz Violet, Disposable Gloves and masks.

Preparation of test fungal

Candida albicans was cultured for approximately 72 hours at 37ºC in Sabouraud Dextrose Broth (SDB). with optical density is 600 microbial culture then meet the standard 0.1 equivalent to the McFarland standard 0.5 ~ 1.5 x 108 CFU/ml).

Antifungal testing

tested with wells on microtiter plate flat-bottom polystyrene 96 with various concentrations of bajakah tampala ethanol extract, i.e 1%, 0.5%, 0.25%, 0.125% w/v. The control used were media control, and infected untreated control (100% growth) for negative control. Fluconazole 1% w/v is a positive control in this test, and DMSO 1% w/v used as vehicle control. Culture plates were Incubation is carried out at 37°C for a period of 72 hours. Optical density readings journey were obtained using a microplate reader at 595 nm.

Test Of Inhibition Of C. albicans Biofilm Formation Mid-Phase (24 Hours) And Mature-Phase (48 Hours) The Usage Of Micro Broth Dilution Approach.

A 96-nicely flat-bottom polystyrene microtiter plate become used to assess the effect of Bajakah tampala ethanol extract at the formation of C. albicans biofilm [13]. a complete of 100 µL C. albicans suspension (10^7 CFU/mL) was added to every nicely of the microtiter plate after which incubated at ± 37°C for 90 minutes for the biofilm attachment phase. After the incubation period, each of the the plate wells have been washed with 100 µL of sterile distilled water three times to get rid of nonadherent cells. a complete of 100 µL media containing ethanolic extract of bajakah tampala with a concentration series (1%, 0.5%, 0.25%, 0.125% w/v) changed into added to every nicely. A medium with out microbial increase became used as a medium control, and a microbial suspension changed into used as a terrible manipulate. 1% w/v DMSO become used as automobile manage, and as high-quality manipulate, Fluconazole at a attention of 1% w/v turned into used in this observe. The plates have been then incubated at 37°C for 48 hours to shape the mid-segment biofilm and for forty eight hours to shape the maturation phase biofilm.

Following biofilm formation, the plate changed into washed with distilled water three instances to detach nonadherent cells, and dried at room temperature for 5 minutes to remove the final water. a total of 125 µL of one% crystal violet solution became introduced to each well to color the shaped biofilm, each dead cells and stay cells, which might be also additives of the biofilm, then incubated at room temperature for 15 minutes. After incubation, the microplate changed into washed below walking water three instances to take away the final crystal violet, and two hundred µL of 96% ethanol changed into added to every nicely to dissolve the formed biofilm. Optical Density (OD) readings had been executed using a microplate reader at a wavelength of 595 nm.

The OD cost is then used to calculate the percentage inhibition inside the following equation:

% inhibition

$$\frac{(\mathrm{OD}_{\text{negative control mean}} - \mathrm{OD}_{\text{test sample mean}})}{\mathrm{OD\ negative\ control\ mean}} \mathrm{X}\ 100$$

The pattern level that may inhibit at the least 50% biofilm formation is considered minimal Biofilm Inhibition concentration $MBIC_{50}$ [14].

Testing of C. albicans biofilm eradication activity

The effect of the ethanolic extract of bajakah tampala was also examined on the preformed biofilm of the C. albicans strain ATCC 10231 using the previously described method [15]. Biofilms were inoculated on microtiter plates as defined above. After incubation at 37oC for 48 h, each of the plate wells were washed with 100 and 100 µL of sterile distilled water 3 times to get rid of nonadherent cells. a entire of 100 µL of media containing ethanolic extract of bajakah tampala with a attention collection (1% w/v, 0.5% w/v, 0.25% w/v, 0.125 % w/v) have become delivered to each well. Plates had been placed decrease again into the incubator at 37ºC for 48 hours. Following the incubation duration, the plates have been washed 3 instances with 200 mL of sterile PBS to remove adhering cells. The staining strategies are defined above. the share of biofilm degradation through the extract tested became calculated, as described earlier than [16].

Scanning electron microscopy (SEM) test

For SEM observations, C. albicans biofilms were formed on the sterile polyvinyl chloride coverslips (with 0.13—17 mm thickness and 22 mm diameter) inside a 12-well microtiter plates (Corning1 Costar1, Sigma-Aldrich, Missouri, USA) in the presence of 0.25 % w/v of ethanolic extract of bajakah tampala for 72 h at 35°C, as described in the previous section. A biofilm grown in the absence of the test drug served as a control. Briefly, the coverslips were removed, washedtwice with sterile PBS (0.1 M and pH 7.2) and placed in a primary fixative

solution [glutaraldehyde 0.15 M 2.5 % (vol/vol) in PBS] at 4 8C for 60 min. The coverslips were subsequently rinsed 2 times with PBS for 5 min, then treated with the secondary fixative (osmium tetroxide OsO4 1% w/v)

for1 h. The samples were subsequently washed with distilled water, dehydrated in an ethanol series (70 % for 10 min, 95 %for 10 min and 100 % for 20 min) and airdried overnight in a desiccator. The coverslip was coated twice with platinum vanadium using a sputter ion (BalTec SCD 005), followed by bonding to carbon doubleside tape for examination by SEM [17].

RESULTS AND DISCUSSION

Antifungal of Bajakah tampala against C. albicans

The results obtained on antifungal testing using microdilution, namely the ethanol extract of Bajakah Tampala was able to provide inhibitory activity against C. albicans by 82.31% ± 0.01 and control activity of Fluconazole drug 88.10% ± 0.01 (Figure 1).

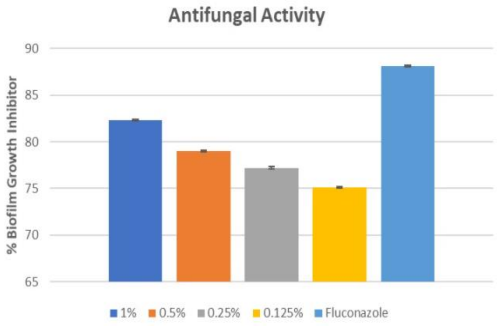

Figure 1. The antifungal effect of the ethanol extract of Bajakah tampala against C. albicans

The results obtained on antifungal testing using microdilution showed that at 1% w/v concentration of ethanol extract Bajakah Tampala was able to provide inhibitory activity against C. albicans as much as 82.31% ± 0.01, whereas Fluconazole as positive control gave 88.10% ± 0.01 growth inhibition, respectively. This result provide evidence that the ethanol extract of Bajakah Tampala has an antifungal activity against C. albicans, and the activity was dose dependent.

Effect of ethanol extract of Bajakah tampala on the mid-phase C. albicans biofilm mono-species (24h).

This study examined the antibiofilm potential of the ethanolic extract of Bajakah Tampala against C. albicans biofilm formation. these consequences imply that the ethanol extract Bajakah Tampala could inhibit as much as 50% of C. albicans biofilm formation (Figure 2).

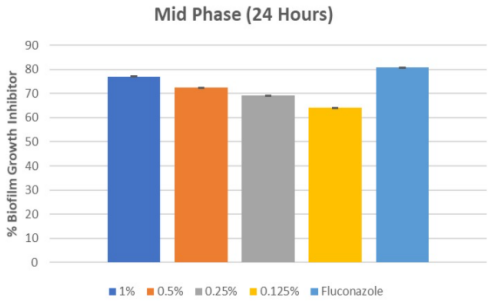

Figure 2. Effect of ethanol extract of Bajakah tampala on C. albicans within the intermediate phase (24 hours) biofilm

The MBIC50 of the ethanol extract of the of Bajakah tampala at concentration of one% w/v against the mid-segment C. albicans was 77.00% ± 0.01, whereas the Fluconazole showed inhibition on C. albicans biofilm formation as much as 80.67 % ± 0.01, respectively.. Form the result above, it has been showed that the same concentration of extract tested (1% w/v) showed weaker activity against biofilm formation inhibition (77.00% ± 0.01) compare to the planktonic (82,31 % ± 0,01), The result obtained indicate that microbes in biofilm forms are more difficult to inhibit than in planktonic., This is likely because microbes in planktonic are single cells, free floating microbes, while microbes in biofilms tend to live together (many colonies), stick, and grow on the surface, and form layered structure encased by an EPS matrix, which make biofilm more resistant to antibiotics and antimicrobials.

Effect of ethanol extract of tampala Bajakah on mono-species biofilm C. albicans maturation phase (48h).

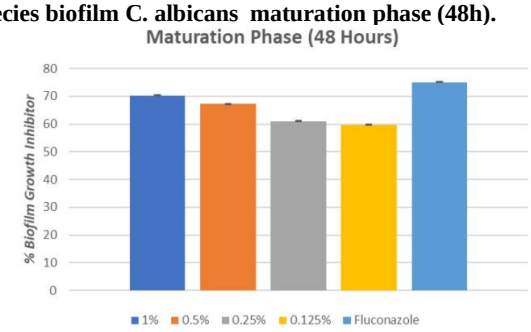

Figure 3. Effect of ethanol extract of Bajakah tampala on mono-species C. albicans in the maturation phase (48 hours)

In this phase, effect of ethanol extract of tampala Bajakah decreased in activity compared to the inhibition phase of planktonic cells and the mid-phase biofilm. This is because, in this phase, the C. albicans biofilm has formed completely so that C. albicans gets fairly strong protection, this visible from the external mucus produced by maturation phase biofilm more than the middle phase, not only that the EPS matrix produced in the maturation phase this was much higher than in the middle phase, where EPS was the protector and nutrient provider for the survival of the C. albicans biofilm so that new colonies continued to form. This is by research [18]. which states that antimicrobial dealers may also have more trouble penetrating biofilm defenses inside the maturation section. Mature biofilms encompass a yeast base with hyphal elements forming a complicated community encased in EPS and a ways from the floor [19], ethanol extract of the of Bajakah tampala 1% w/v gave an inhibitory activity in the ripening phase of 70.31% ± 0.01, while the activity (Fluconazole), which was used as a control drug, showed a better inhibitory activity of 75.10% ± 0.01 (Figure 3). This shows that in the maturation section (48 hours), the ethanolic extract of the Bajakah tampala can offer 50% interest.

These results provide evidence that the longer the biofilm growth time, the more the matrix arrangement produced, and the stronger and more complex the biofilm structure formed. The test compound or drug control decreased activity in inhibiting the biofilm. This result is reinforced by Bjarnsholt's (2013) statement and Hamzah's (2018) [20], who state that antimicrobial dealers can have extra trouble penetrating biofilm

defenses within the maturation section. The interaction among species causes colonization and infection dynamics, in addition to a number of other responses [21]

Biofilm Eradication activity of ethanol extract of Bajakah tampala against C. albicans biofilm.

Using the crystal violet staining, we have found that at concentration of 1% w/v the ethanol extract of Bajakah tampala could eradicate C. albicans biofilm by 65.21% ± 0.01, whereas Fluconazole degrade as much as 69.11% ± 0.01 of C. albicans biofilm, accordingly (Figure 4).

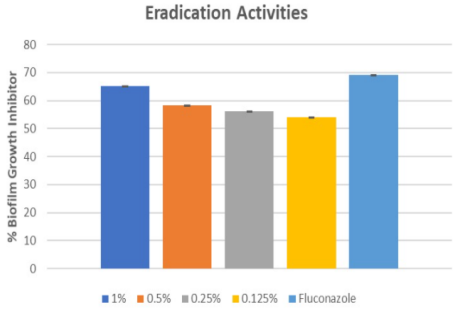

Figure 4. The activity of the ethanol extract of the of bajakah tampala in eradicating C. albicans biofilm

At the same concentration, the activity of the extract tested in degrading C. albicans established biofilms was weaker (65.21% ± 0.01) than the activity in inhibit C. albicans biofilm formation (77.00% ± 0.01). The decrease in the activity of the ethanol extract of Bajakah tampala in eradicating C. albicans biofilm is thought to be due to the more complex and structured of preformed biofilms. Biofilm microbes formed a highly structured cell as well as thick and dense EPS substances surrounding the biofilm cells, thatprotect the microbes in the biofilm from antimicrobial and antibiofilm agents. According to Stewart and Costerton (200) [22] antibiotic therapy can eliminate or reduce planktonic bacteria, however bacteria in the biofilm are more persistent. When treatment with antibiotics is completed, the biofilm will form planktonic cells again, which results in acute infection.

Scanning electron micrograph (SEM) result of untreated C. albicans biofilm

The activity of Bajakah tampala ethanol xtract in inhibit and breakdown C. albicans biofilm was confirmed by SEM analysis. The results confirmed that untreated C. albicans had a high cellular density inside the EPS Matrix protecting C. albicans (figure 5). This shows that a biofilm has formed.

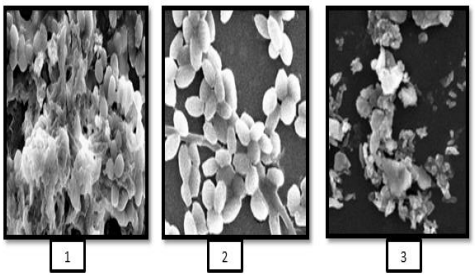

Figure 5. Scanning Electron Microscopy (SEM) photo of C. albicans treated with zero.five% w/v ethanol extract of Bajakah tampala (1,2: earlier than remedy, three: after remedy). Ethanol extract of Bajakah tampala at awareness of zero.five% w/v can purpose lysis in C. albicans biofilms cells and the mobile density decreases (Figure 5).

The biofilm matrix serves as a hyperlink among adhesive and cohesive interactions, which provides mechanical stability to the biofilm, controls cell dispersion of the biofilm, and can also act as a nutrient source provider for cell communication [23]. The C. albicans biofilm matrix additionally acts as a protecting cellular biofilm and a primary barrier defensive biofilm cells all through assaults from the immune system and antifungal drug treatment

Scanning electron micrograph (SEM) result of C. tropicalis administrated by 0.5% w/v ethanol extract of Bajakah tampala

Image from SEM (figure 5) shows that the ethanol extract of Bajakah tampala was proven to disturb C. albicans biofilm because the active compound could attack the EPS matrix of the biofilm. On the other hand, decreased cell attachment, density, and lysis were observed.

CONCLUSION

The ethanolic extract of the Bajakah tampala has an antifungal and antibiofilm activity against C. albicans .and also capable to eradicate C. albicans biofilms. The ethanolic extract of the Bajakah tampala is capability to be developed as candidate for antifungal and antibiofilm of C. albicans .

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Ethical Approval: This study did not use experimental animals so it does not require approval from the ethics committee.

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Conflict Of Interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Informed Consent: The research focused on the biofilm of C. albicans, one of the microbes that causes wound infection with diabetic ulcers on the foot, using a plant from the Indonesian Borneo Island, Bajakah Tampala.

Authorship

H. Hamzah : main idea of research, biofilm testing, final approval, data analysis

S.U.T Pratiwi : main idea of research, biofilm testing

A. Jabbar : Plant determination and extract manufacture

E. Nandini : draft writing

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