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SCANNING ELECTRON MICROSCOPY OF *CANDIDA ALBICANS* CELL WALL ON TOOTH SURFACE AFTER FLUCONAZOLE-TREATED AND INCUBATED WITH GUAVA (*PSIDIUM GUAJAVA L*)

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Abstract

The *Candida albicans* cell wall is a target of fluconazole. The content of guava (*Psidium guajava L*) known have contribute factors to resistance of fluconazole. Objective: characterize the surface alterations and the general shape changes of candida cells after in vitro exposure to fluconazole and guava. Material and Methods: six tooth specimen groups: Cells exposed without fungicidal doses (Negative Control), Positive Cells exposed to maximal fungicidal doses of Fluconazole (100µg/ml) (Control), Guava (G), Guava+Fluconazole susceptibility (GFS), Guava+Fluconazole Susceptible-Dose Dependent (GFSDD), Guava+Fluconazole dose Resistance (GFR). Each group mixed with 10 ml artificial saliva, 2 ml glucose 2%, and 2 ml *C. albicans*. Fluconazole doses based on NCCLS approved M27-A. Scaninng Electron Microscopy conducted after 15 days incubation at Faculty of Veterinary University of Syiah Kuala and Faculty of Engineering University of Indonesia. Results, SEM of Negative Control presented smooth of blastospores; small clusters of interconnected cells, spherical to elongate in shape, lying down, polar buds, and hyphae on dental surface. SEM of Positive Control and Guava generally presented: wrinkled of blastospores, rough surfaces, cells fewer than Negative Control, and lying apart. Positive Control showed spherical to elongate in shape and Guava with spherical in shape presented more wrinkled than Positive Control. SEM of GGF and Guava+Fluconazole Susceptible-Dose Dependent presented wrinkled of blastospores, rough surfaces, decreased in number, spherical to elongate in shape, lying apart, and hyphae on surface. SEM of Guava+Fluconazole dose Resistance presented wrinkled of blastospores but more smooth than others, elongate in shape; bud's orientations penetrate to dental tissue. Conclusion: Guava improving the integrity of cell wall of *Candida albicans* and contributed to resistance of *Candida albicans* to fluconazole.

Keywords: *Candida albicans*, Guava, Fluconazole, *Scanning Electron Microscopy*

INTRODUCTION

Candida albicans is a normal microflora in the oral cavity.[1] *Candida albicans* is a frequent microorganism that found in digestive tract of healthy individuals. On the other side, *Candida albicans* is an important opportunistic pathogen of immunocompromised individuals causing diseases that range from superficial mucocutaneous infections to life-threatening systemic candidiasis.[2] Oropharyngeal candidiasis (OPC) is the most frequent opportunistic fungal infection among human immunodeficiency virus (HIV)-infected patients.[3]

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A Comensalism become pathogenic organism is highly dependent on adhesion factors.[4] *Candida albicans* adhesion in an inert surface that has been widely reported is the adhesion to buccal epithelial cells (BEC), surface of prosthetic devices, artificial ingredients hydroxy apatite, and tooth.[5] Data and explanation of the adhesion and the colonization of *C. albicans* on the tooth surface by Scanning Electron Microscopy (SEM) examination are limited.[6]

Cell membrane of *C. albicans* has sterol that serves as the workings set of several enzymes such manan synthase, chitin synthase, glucan synthase, and ATPases that known play an important role in synthesis of *C. albicans* cell wall.[7] Ergosterol is sterols which is an important regulator of *C. albicans* structure and components. Ergosterol is responsible for various cellular functions in membrane structure such as fluidity and permeability as well as the cholesterol functions in mamalia cell.[7,8] Both ergosterol and cholesterol synthesized on the same pathway. Some studies reported that there is a possibility of cholesterol can replace the function of ergosterol.[7,9]

Ergosterol biosynthesis plays a critical role as a target of antimycotics. One of antimycotics that inhibit ergosterol biosynthesis is fluconazole. Resistance of *Candida albicans* to azole may occur due to changes in cell membrane composition, especially the components of sterols in the cell membrane that decreasing drug sensitivity. [10]

Today, guava (*Psidium guajava L*) is known as one of famous beverage for human health. Guava has triterpenoid that capable to binding cholesterol.[11] The study mentioned that pathogenic microorganisms such as *Trypanosoma* and *Saccharomyces cerevisae* can utilize external cholesterol in anaerob conditions.[12] Other factors that can contribute to the resistance of *C. albicans* to fluconazole are iron and calcium that also found on guava. These substances reported increasing the virulence of *C. albicans* to host epithelium.[13,14] The purpose of this study is to characterize with the aid of Scanning Electron Microscopy the surface alterations and the general shape changes of *C. albicans* cells after in vitro exposure to antimycotic fluconazole and guava.

MATERIAL AND METHODE

Six tooth specimen groups: Positive Control (K+), **Cells exposed without fungicidal doses** (Negative Control), Guava, Guava+Fluconazole susceptibility, Guava+Fluconazole susceptible-Dose Dependent, Guava+Fluconazole dose Resistance. Each group mixed with 10ml artificial saliva, 2ml glucose 2%, and 2ml *C. albicans*. Fluconazole doses based on National Committee for Clinical Laboratory Standards approved M27-A3[15] Scanning Electron Microscopy conducted after 15 days incubation. Research conducted at Faculty of Veterinary University of Syiah Kuala and Faculty of Engineering University of Indonesia. *Candida albicans* suspension was prepared by inoculates 1 osse that contain pure cultures into 10 ml of peptone and compared to 0.5 Mc. Farland (equivalent to $1.5x10^8$ CFU/ mL). All specimens incubated at 37°C for 24 hours.

RESULT

Cells exposed without fungicidal doses presented smooth of blastospores; small clusters of interconnected cells, spherical to elongate in shape, lying down, polar buds, and hyphae on dental surface (Figure1). Cells exposed to maximal fungicidal doses of Fluconazole (100µg/ml). Generally presented wrinkled of blastospores, rough surfaces, cells fewer than Cells exposed without fungicidal doses, and lying apart, spherical to elongate in shape (Figure 2). Cells exposed to guava: generally presented: wrinkled of blastospores, rough surfaces, cells fewer than Cells exposed without fungicidal doses, and lying apart, spherical in shape and presented more wrinkled than Cells exposed to maximal fungicidal doses of Fluconazole (100µg/ml) (Figure 3). Cells exposed to susceptible doses of Fluconazole (8µG/ml) +Guava: presented wrinkled of blastospores, rough surfaces, decreased in number, spherical to elongate in

shape, lying apart, and hyphae on surface (Figure 4).

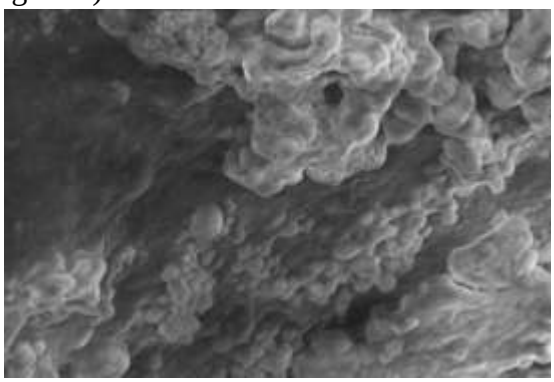

Figure 1. Cells exposed without fungicidal doses presented smooth of blastospores; small clusters of interconnected cells, spherical to elongate in shape, lying down, polar buds, and hyphae on dental surface. SEM Magnification 3000 X

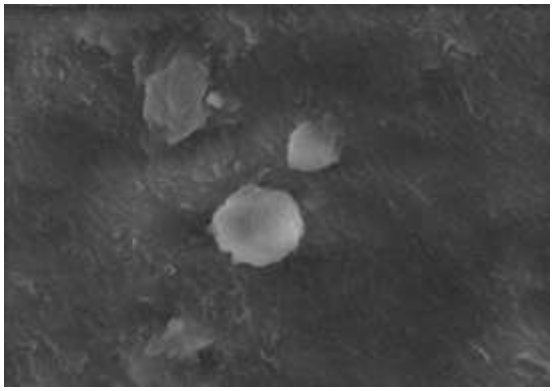

Figure 2. Cells exposed to maximal fungicidal doses of fluconazole (100µg/ml) generally presented wrinkled of blastospores, rough surfaces, cells fewer than negative control, lying apart, spherical to elongate in shape. SEM Magnification 5000X

Figure 3. Cells exposed to guava presented: wrinkled of blastospores, rough surfaces, cells fewer than Control-, and lying apart, spherical in shape, and more wrinkled than Possitive Control. SEM Magnification 7000X

Cells exposed to *susceptible doses dependent/ optimum (*32µg/ml of Fluconazole+Guava: presented wrinkled of blastospores, rough surfaces, decreased in number, spherical to elongate in shape, lying apart, and hyphae on surface (Figure 5).

Cells exposed to *Resistance* dose 64µg/ml of Fluconazole+Guava: presented wrinkled of blastospores but more smooth than others, elongate in shape; bud's orientations penetrate to dental tissue (Figure 6).

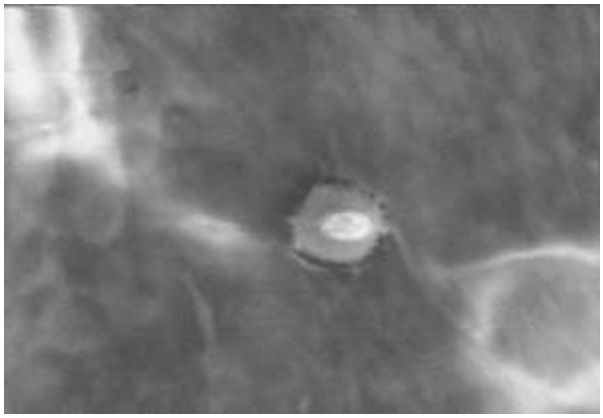

Figure 4. Cells exposed to susceptible doses of fluconazole (8µG/ml) +Guava: presented wrinkled of blastospores, rough surfaces, decreased in number, spherical to elongate in shape, lying apart, and hyphae on surface. SEM Magnification 7000X

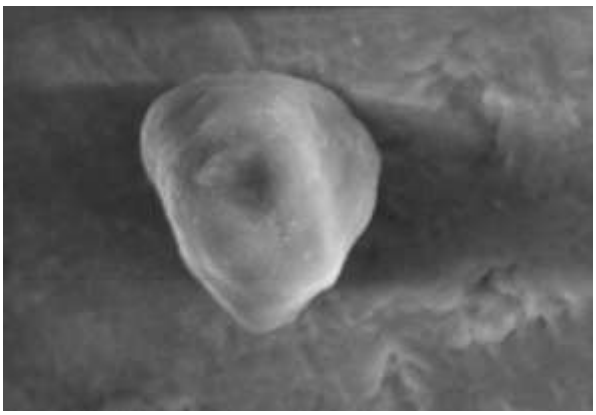

Figure 5. Cells exposed to *susceptible doses dependent/ optimum (*32µg/ml of Fluconazole+Guava: presented wrinkled of blastospores, rough surfaces, decreased in number, spherical to elongate in shape, lying apart, and hyphae on surface. SEM Magnification 7000X

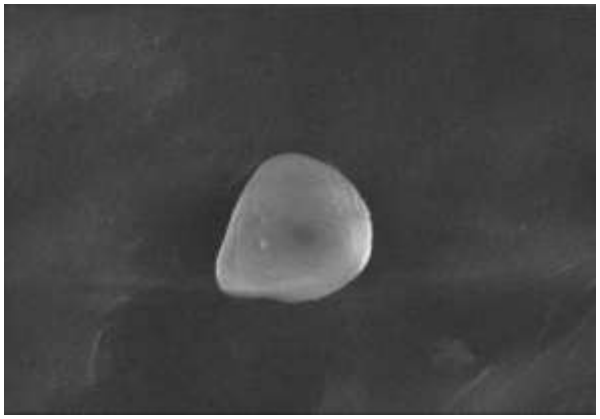

Figure 6. Cells exposed to *Resistance* dose 64µg/ml of fluconazole +Guava: presented wrinkled of blastospores but more smooth than others, elongate in shape; bud's orientations penetrate to dental tissue. SEM Magnification 7000X

DISCUSSION

Scanning Electron Microscopy image of *Candida albicans* cells that exposed to rluconazole presented rough surface, wrinkled, do not form hyphae, and blastopora cells appear more spherical. This result is similar to Bachmann et al.[16] that reported in vitro antimycotics into candida affected cells look rough, wrinkled and decreasing of hyphae form. Similar to that report, Samaranayake et al.[17] reported that the morphology of *C. albicans* that exposed to antimycotics azole affecting cell's wall atrophy. Wrinkles in the cell wall due to deposited material of cells membrane that deposited between the cytoplasm and cell walls, or inside the cell wall affected cell more spherical or bubble-like cells. Other research also reported that after exposure to substances that interfere to cells growth, cell presented lying position and change of buds.[18] Target of rluconazole either the formation or the function of ergosterol that known as important component of the fungal cell membrane.[10] Scratch of structure and function of fungal cell membrane ultimately inhibit the growth and cell morphogenesis.[19] Fluconazole is also disrupting the fluidity of the cytoplasm and cell walls by inducing of permeability changes. This condition have affected osmotic imbalance in the cell of *C. albicans* that contribute to maintain the integrity of the structure and function of *C. albicans* cell walls.

Guava (*Psidium guajava L*) that usually consumed by people positively contributes to increase of resistance of *C. albicans* to fluconazole. Factly, fluconazole will decrease ergosterol level, but when this antimycotic exposed to guava, inhibition of ergosterol component in plasma membrane will recompense through utilizing of extracellular sterols.[10] Guava that exposed to fluconazole facilitated the possibility of candida to improving sterol transcription factors to regulate both expression and intake of extracellular sterol.[12,20,21] The main substance that contributes cell of candida to survive through bind extracellular sterol in guava is triterpenoid.[11] Triterpenoid bind to the sterol of *C. albicans*. The nature of nonpolar triterpenoid easily penetrate into cell membrane or organelles on the hydrophobic side that form micell structure, ie the bonds between nonpolar compounds (triterpenoids) with nonpolar part of the cell membrane that causing leakage in membrane of candida.[22]

However, the permeability disturbance can be compensated by *Candida albicans* cells through a variety of defense mechanisms. One important defense mechanism through kinase pathway that activates the cell wall repair genes by captivating extracellular calcium and phosphates through the plasma membrane that support regulate the cells cycle and cells morphogenesis.[16] Beside, SEM image presented that candida change morphology of cells and penetrate into another tissue to let cells have nutrients and survive.[23] *Candida albicans* cell morphology is influenced by various factors, including nutrition.[24] *Candida albicans* is dentinophilic organism that capable to make dentin and extracellular calcium as a source of nutrients.[25] Candida maintained cells's integrity through penetrate to tooth tissue without induction of hyphae on surfaces. Both factors such as condition that lack of nutrient and tooth as adhesion media of candida were confirm that induction of

hyphae occasionally not just influenced by chemical factors but rather due to physical environment.[26] Calcium is an essential component to several cell components such as endoplasmic reticulum, mitochondria, and vacuoles. These organelles were important to regulate the availability of calcium through calcineurin that known as defend's factor for cells to against undesirable conditions such as interference of cell membrane. Azole exposure affected calcium out through plasma membrane or intracellular components into cytosol that changes the permeability.[14] Scanning Electron Microscopy image also confirm that addition of guava will affect cell of *C. albicans* more spherical and several cells presented more smooth surfaces than fluconazole treatment. Lim et al.[27] stated that the presence of secondary metabolites that found in plants affect the activity of cytology and cell morphology of *C. albicans*.

Iron that contained in guava also plays an important role in cell regulation and cell wall biosynthesis of *C. albicans*. Iron is an essential nutrient in the growth of cells *C. albicans* needs these substances obtained even invasion of candida into the vascular tissue.[24] Iron is known as substance that supporting the virulence of *C. albicans* in membrane fluidity through decreasing diffusion of antimycotics agent.[13] Other substance in guava that contributes to candida cells to compensate is carbohydrates. Source of carbon that obtains from carbohydrates affects the form of budding; bud morphology, surface topography and morphology of colonies.[22] This occurrence are confirm in SEM images that show the topography surface of cell walls are smooth, no major damage, and bud's orientation leads to tooth surface. These substances mediate changes sterol composition in cell membranes to affected membrane permeability eventually antimycotics agents were inhibiting it.[28]

CONCLUSION

Guava (*Psidium guajava Linn*) improving the integrity of cell wall of *Candida albicans* and contributed to resistance of *Candida albicans* to fluconazole.

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