POTENTIAL LEAF EXTRACT *Orthosiphon aristatus* AS GROWTH INHIBITOR OF *Candida albicans*

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ABSTRACT

The use of antifungal ketoconazole in treatment of candidiasis should be limited due to side effects endocrine and narrow therapeutic range. This study aims to compare the antifungal potential between Orthosiphon aristatus leaf extract and ketoconazole against the growth of Candida albicans. C. albicans growth inhibition test is done by giving the antifungal ketoconazole with 2% concentration and Orthosiphon aristatus leaf extract with 25%, 50% and 100% concentration. Growth measurements directly used hemocytometer counting chamber. Live and dead cells were counted every 3 hours for 48 hours of incubation. The growth curve and death cell curve showed ketoconazole more potent in inhibited the growth of C. albicans when compared with Orthosiphon aristatus leaf extract. Orthosiphon aristatus leaf extract has potential as an antifungal to C. albicans with the most optimal inhibitory concentration is 25%.

Keyword: ***Anfifungal, Orthosiphon Aristatus Leaf Extract, Ketoconazole, c. Albicans.***

INTRODUCTION

Candida albicans is a microscopic fungi that pathogenic in the human body. *Candida sp* is normal flora in various human organs. However, disruption of the normal flora balance for a variety of predisposing factors, can lead to an expanding population of *Candida sp* (Budimulja, 2001). *Candida sp* is the most common species cause of fungal infections. *Candidiasis* can occur in almost all organs of the body (Pappas, et al, 2004). The most common site of infection in the mouth (rash) and / or the vagina (vaginal candidiasis). Candidiasis is usually treated by synthetic drugs, one of them is ketoconazole. However, the use of ketoconazole has been reported to have side effects such as gastrointestinal, pruritus, liver dysfunction, myalgia, headache, and vomiting (Heel et al, 2012). Therefore, we need an alternative treatment for candidiasis, one of them is natural treatment.

Orthosiphon aristatus leaves can be used as an alternative treatment of candidiasis caused by *C. albicans*. *Orthosiphon aristatus* leaves potential as a diuretic drugs (Adam et al, 2009), while in Indonesia, *orthosiphon aristatus* known as a traditional treatment for stones gall bladder, bladder and urinary infections, vaginal discharge (one effect of candidiasis) and urinary stones, *Orthosiphon aristatus* leaves contain essential oils, orthosiphonglikosida, saponin, sapofonin, and potassium salts. Essential oil is a natural substance that has been known to have antimicrobial activity. Saponins often called natural detergent, a foaming solution and are classified into triterpenoid and steroid saponins. Both of these compounds have anti-inflammatory, analgesic, cytotoxic, and to be antimicrobial. While flavonoids are the class of phenolic compounds also have the ability as an antimicrobial.

This study aimed to compare the potential antifungal between *Orthosiphon*

aristatus leaf extracts and ketoconazole for inhibiting the growth of *C. albicans*. Specifically, the study will obtain information *Orthosiphon aristatus* leaf extract optimal concentration in inhibiting the growth of *C. albicans*, thus encouraging further research in the manufacture of natural medicine from *Orthosiphon aristatus*, especially for the treatment of candidiasis.

METHODS

Comparison research between *Orthosiphon aristatus* leaf extracts with ketoconazole on the growth of *C. albicans* was an experimental study. This study makes manipulation of research objects, namely antifungal as an independent variable in order to observe the growth of *C. albicans* (dependent variable). Ketoconazole concentration used was 2% in the medium. While *Orthosiphon aristatus* leaf extract concentration used was 25%, 50% and 100%. The use of different concentrations *Orthosiphon aristatus* is intended to determine the most optimal concentrations to inhibit the growth of *C. albicans*. In addition, there is a control group, without antifungal activity, which uses as a reference medium that show normal cell growth of *C. albicans*.

Isolation and identification is performed to determine whether the microbes to be used is *C. albicans*. The main stage is the identification of microbial characterization test in order to know the character of microbes for further profile matching with the character of *C. albicans* in the book The Yeast, A Taxonomic Study Fifth Edition (Kurtzman et al, 2011). Characterization includes characters colony morphology, cell morphology, physiology, and biochemistry (fermentation ability against some types of carbohydrates) observations.

Orthosiphon aristatus leaves extraction performed to obtain leaf extract containing chemical compounds that have the potential as an antimicrobial like flavonoids, saponins and essential oils. Chemical compound to be derived from the leaves of *Orthosiphon aristatus* not stand by heating, so the method used in the extraction is maceration. Maceration is a filtration process of chemical compound simply by immersing the bulbs at room temperature using ethanol solvent so that the material becomes soft and soluble. By soaking the plant samples in the solvent will break the cell wall and cell membrane due to the difference pressure between inside and outside of the cell, so that the solvent will penetrate the cell wall and into the cavity of the cell that contains the active substance and the active substance will dissolve. Solvents used in the extraction is 70% ethanol, because ethanol is a universal solvent that can optimize expenditures flavonoids and saponins. 78.22 grams of crude *Orthosiphon aristatus* leaves extracted with 1300 ml of 70% ethanol produces leaf extract then diluted to obtain concentration that needed in research, that are 25%, 50% and 100%.

The media used to grow the isolation of *C. albicans* is Saboraud Dextrose Broth (SDB) media. SDB is a liquid medium with peptone and dextrose composition (Atlas RM, 2010), it is made by mixing mycological peptone, glucose, distilled water, and chloramphenicol. Treatment media prepared containing ketoconazole 2% and *Orthosiphon aristatus* leaf extract 25%, 50% and 100%.

Data collection technique is the antifungal test with method to counting live and dead cells directly using hemocytometer count tools. In order to distinguish live and dead cells, used 0.1% methylene blue dye. Calculations performed once per three hours of each treatment for 48 hours of incubation. Results of counting the number of cells per ml of antifungal test medium every three hours made a growth curve and death cell

curve by calculating the average of cells of three replications. From the curve it will be known comparison the antifungal capabilities between ketoconazole and *Orthosiphon aristatus* leaf extract. It is also can determine the optimal concentration of *Orthosiphon aristatus* leaf extract in inhibiting the growth of the C. *albicans*.

RESULTS AND DISCUSSIONS

Cell growth curve (Figure 1) and cell death curve (Figure 2) on control and treatment media provide antifungal potential comparision of ketoconazole 2% and *Orthosiphon aristatus* leaf extract (25%, 50% and 100%). There is inhibition of the growth of *C. albicans* in the media with ketoconazole 2%. Ketoconazole 2% more effective in inhibiting the growth of microbes compared with *Orthosiphon aristatus* leaf extract 25%. *Orthosiphon aristatus* leaf extract has antifungal potential to inhibit the growth of *C. albicans* and there are inhibition differences on different concentration of leaf extract. The increase in the concentration of leaf extract not further inhibit cell growth, but instead promotes cell growth. From the three concentrations are given as treatment, 25% *Orthosiphon aristatus* leaf extract is the most optimal in inhibiting the growth of *C. albicans*, followed by 50% and 100% leaf extract concentration.

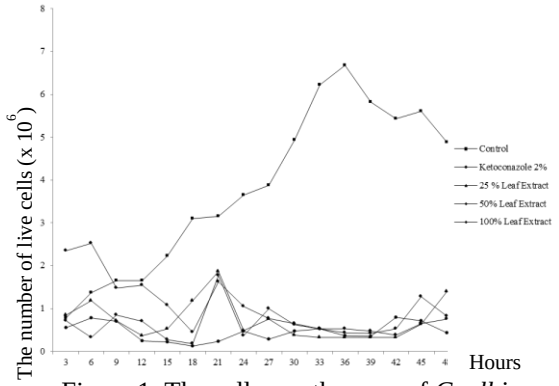

Figure 1. The cell growth curve of *C. albicans* based on quantification data of live cells on control and treatment media

Orthosiphon aristatus leaf extract and ketoconazole both contain chemical compounds that can affect the growth of *C. albicans*, both of them can kill or inhibit the growth of cells, but has different antifungal compound and capabilities mechanisms. The mechanism of antifungal ketoconazole is by inhibiting the enzyme cytochrome P450 14-demethylase. This enzyme is needed in the synthesis of cell membranes to convert lanosterol into ergosterol. Ergosterol which does not form would degrade the integrity and cell membrane permeability. The cell membrane acts as a selective permeability barrier, has role in active transport function, and controls the internal composition of the cell. If the function of cell membrane integrity undermined, macromolecules and ions out of the cell, then the cell is damaged or occurring cell death (Brooks et al, 2015).

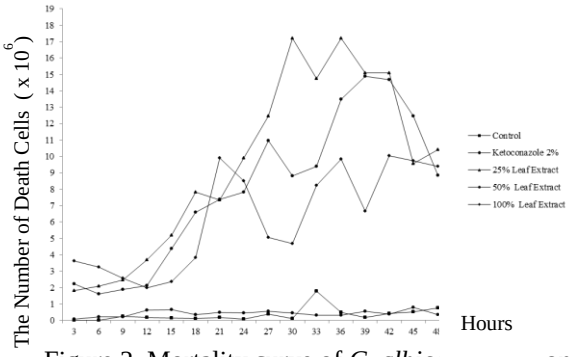

Figure 2. Mortality curve of *C. albicans* based on quantification data of death cells on control and treatment media

There are several possibilities mechanism of *Orthosiphon aristatus* leaf extract in inhibiting the growth of *C. albicans*. *Orthosiphon aristatus* leaf extract contains several active chemical compounds that could potentially inhibit cell growth, such as saponins, flavonoids and essential oils. From various studies on the mechanism of action of some antimicrobial active compounds, the active compound has a potential and mechanism difference as an antimicrobial, but the third target of the active compound is almost the

same, ie the cell wall and cell membrane. Saponin proven antifungal for *C. albicans* and *Cryptococcus neoformans* also mollusicidal on *Biomphalaria alexandria* (Ekabo et al, 1996). The mechanism of saponin in inhibiting the growth of *C. albicans* is the ability to lower the surface tension of the cell membrane. Saponins destabilizing *C. albicans* cell membrane, thus causing damage to the cell membrane. Damage to the cell membrane resulting in the release of various important components of the cell, ie proteins, nucleic acids, nucleotides, and others, in other words, the cells undergo lysis. The flavonoid compound is one of the largest natural phenols. Flavonoid compounds found in some plants proved to be antifungal, antiviral, and antibacterial (Orham et al, 2010) with the inhibition mechanism of damaging the cell walls, changing the permeability of the cell membrane, the cell's protein denaturation, and damage the metabolism system in cells by inhibiting the intracellular enzyme. Essential oils shown to have antibacterial activity (Marliani L, 2012) and antifungal (Dian M, 2008). *Orthosiphon aristatus* leaf essential oil is also composed of phenol compounds. The antimicrobial activity of phenolic compounds have been described above as an activity of flavonoids.

In this study, an increased concentration of *Orthosiphon aristatus* leaf extract no further streamline the active compounds to inhibit the growth of *C. albicans*. However, the use of the extract concentration of the lowest (25%) is the most effective in inhibiting the growth of *C. albicans*, it is seen from the highest levels of cell death in the media with *Orthosiphon aristatus* leaf extract 25%. This is possible because several things such as giving *Orthosiphon aristatus* leaf extract with a high concentration (50% and 100%) resulting in cell resistance and the interaction of active and non-active compounds in the extract of leaves of *Orthosiphon aristatus* weaken the potential antimicrobial. The number of chemical compounds contained in *orthosiphon aristatus* will not necessarily be more effective inhibit the growth of *C. albicans*, because between the chemical compound will occur interaction that might weaken the antimicrobial mechanism. Increasing concentrations of non-active compound can reduce the concentration of active compounds that dissolved in the media, which in turn led to a decrease in the activity of the active compound (essential oils) (Ardani et al, 2010). In addition, the presence of organic materials in a mixture of antimicrobial agents may lead to the incorporation of antimicrobial substances with organic material forms a precipitate that antimicrobial no longer possible to bind microorganisms and accumulation of organic material on the surface of microbial cell becomes a protector that will disturb the contact between the antimicrobial agent with the cell (Widyarto, 2009).

From the discussion of antimicrobial active compounds mechanism in the leaves of *Orthosiphon aristatus*, the inhibition of the growth of *C. albicans* by ketoconazole is greater than the inhibition of *Orthosiphon aristatus* leaf extract. *Orthosiphon aristatus* leaf extract has phases in inhibiting cell growth, starting with the destruction of cell walls, cell membranes, and proteins in the cell, so in inhibiting the growth or cause cell death it takes longer than ketoconazole. Ketoconazole works more specifically, directly in cytochrome P450 which is required for the synthesis of ergosterol and inhibits the biosynthesis of triglycerides and phospholipids. Therefore, in the formation of cell walls, ketoconazole already is fungicidal (kill) against *C. albicans*. While *Orthosiphon aristatus* leaf

extract only fungistatis or inhibit growth against the microbe.

CONCLUSION

Ketoconazole inhibit the growth of *C. albicans* greater than *Orthosiphon aristatus* leaf extract. *Orthosiphon aristatus* leaf extract potentially inhibit the growth of *C. albicans* with the most optimal inhibitory concentration is 25%. Therefore, *Orthosiphon aristatus* leaves can be used as an alternative natural medicine for candidiasis and require further research to draw on *orthosiphon aristatus* as candidiasis drugs.

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