Membrane cell disruption of *Candida albicans* by Masoyi bark essential oil

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**ABSTRACT**

Essential oils distilled from the bark of *Massoia aromatica* Becc. (Masoyi) has been known from our previous research as a potential anti-candidiasis towards both planktonic and biofilm states. This study aims to determine Masoyi essential oil’s antifungal mechanism of action towards *Candida albicans* ATCC 10231. Observation of the antifungal mechanism towards *C. albicans* was performed by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) following the Masoyi essential oil application in sub-minimum inhibition concentration, in comparison to untreated cells as the control. Considering that both planktonic and biofilm of *C. albicans* were influenced, the effect on farnesol production was also observed using thin layer chromatography of the microbial supernatant. Following SEM and TEM results, the effect on membrane permeability seems to play a role in the antifungal activity. To support the result, an experiment using influx Propidium Iodide Test was performed. TLC densitometry and GC-MS analysis showed that C-10 Massoialactone is a major constituent of Masoyi oil. Observation of *C. albicans* cells by using SEM showed cell shrinkage, which may be due to disruption in membrane cell permeability. TEM confirmed the membrane cell disruption, which causes cytoplasmic leakage. The result of the propidium iodide influx test confirmed the damage to membrane permeability. The decrease in farnesol production was observed, which may play a role in the antibiofilm activity of the essential oil.

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**INTRODUCTION**

*Candida albicans* is a polymorphic fungus living as a part of human commensal microflora, mostly found in the mouth cavity and digestive tract ([Mayer et al., 2013](#)). However, in the last three decades, the case of *C. albicans* infection has increased rapidly in immune-compromised patients such as those who have HIV/AIDS ([Samaranayake et al., 2002](#)). Medical treatment with available systemic antifungi gave a positive response, yet the side effects such as fever, vomiting, muscle spasm, and hypotension have limited the therapy. Moreover, [Al-Attas and Amro (2010)](#) reported that diabetes mellitus patients having candidiasis are treated with azoles such as fluconazole, ketoconazole, econazole and miconazole. These issues raise the need for new, more effective antifungi with few side effects.

Medicinal plants offer an alternative source of a
new chemical entity which may serve as an anti-infected. One of the plants commonly used in traditional medicine is *Massoa aromatica* Becc (Lauraceae) known by its local name, Masoyi (Rali *et al.*, 2007). The essential oil distilled from the bark has been reported as an effective anti-microbial by inhibiting the growth of the planktonic cell and biofilm of microbial cells (Hamzah *et al.*, 2018, 2020; Pratiwi *et al.*, 2015) and has an immunomodulatory potential of which the C-10 massoialactone plays a role as the active compound (Hertiani *et al.*, 2016; Permanasari *et al.*, 2017). The Masoyi bark essential oil contains lactone compounds of C10 and C12 while C14 (5,6-dihydro-6-nonyl-2H-piran-2-on) was reported in the hard wood (Rali *et al.*, 2007). Our research has revealed that Masoyi oil provides an inhibitory effect on biofilm formation and degradation of multispecies biofilms (unpublished result). In this recent result, we explore the effect of the Masoyi oil on *C. albicans* membrane. We believe that the effect on the cell membrane may interfere with the antifungal activity and further affecting the biofilm formation of the fungi.

**MATERIALS AND METHODS**

*Massoa aromatica* barks were collected from Sorong (Papua, Indonesia). Sample identification was performed by Mr. Djoko Santos (Laboratory of Pharmacognosy, Faculty of Pharmacy UGM). *C. albicans* ATCC 1023 was used for the assay. C-10 massoialactone was isolated from the essential oil, and the structure elucidation was confirmed in the previous study (Permanasari *et al.*, 2017). Other materials used were farnesol standard (Sigma-Aldrich, Germany) and Propidium iodide (Sigma-Aldrich, Germany).

**Sample preparation**

The dried-pulverized barks were distilled by water-steam distillation method to yield the essential oil. TLC and Gas-Chromatography Mass Spectrometry (GCMS) were performed on the oil to evaluate the content of C-10 massoialactone as the active ingredient as well as to observe the profile of the oil chemical content. GCMS was performed at the Organic chemistry laboratory, Faculty of Mathematical and Life Sciences UGM, while the TLC scanner was provided by the Phytochemistry laboratory, Faculty of Pharmacy, UGM.

**Anti candida assays**

The oil was first evaluated its inhibitory effect on biofilm growth of *C. albicans* to determine the MBIC₅₀ value (minimum sample concentration to reduce biofilm by 50%). Masoyi oil in concentration series of 1%, 0.5%, 0.25%, 0.125% v/v were tested according to Pierce *et al.* (2008). The treatments were divided into three groups, i.e., cells without treatment, cells with the treatment, and another group as a control using Nystatin. The MBIC₅₀ value of oil was used to evaluate the effect of the oil on cell membranes using Influx Propidium iodide (PI) (Lee and Lee, 2014). Transmission electron microscopy (TEM) was performed according to Sangetha *et al.* (2009) while Scanning electron microscopy (SEM) was conducted by the modification of methods described by Hess *et al.* (2012); Hamzah *et al.* (2019); Pelzer *et al.* (2012).

The effect on farnesol production was performed according to the method described by Tashiro *et al.* (2012). The test of farnesol production following sample application was observed by using TLC scanner showed the result of TLC with the observation under a lamp of UV 254 nm, in which the concentration of farnesol standard was prepared as follows, 0.5 μL, 1 μL, 2 μL, and 4 μL.

**RESULTS AND DISCUSSION**

The oil yield obtained from the distillation process was 0.76% v/w. Thin-layer chromatography analysis was done to obtain the chemical profile of masoyi oil compounds. The visualization using UV254 lamp and visible lamp after the application of anisaldehyde−H₂SO₄ spray reagent showed three spots with hRF of 37, 50, 70. hRF 37 was identified as C-10 massoialactone (Figure 1). The result of GC-MS analysis (Figure 2). Showed two peaks, of which 95.68% is identified as C-10-massoialactone (SI 93%) and 4.32% as C-8 massoialactone (SI 90%) (NIST2.LIB and WILLEY229).

Note: Stationary phase: silica gel F254; mobile phase: toluene-ethyl acetate (93:7 v/v), elution path 8 cm. Visualization method: (A) anisaldehyde−H₂SO₄, observed under visible light; (B) anisaldehyde−H₂SO₄, observed under UV 366 nm (C) UV 254 nm, before spraying

The result from *C. albicans* middle phase (24 h) biofilm formation inhibition test showed a decrease in the formation of *C. albicans* biofilm following the increase of the Masoyi oil concentration (Figure 3). Nystatin was used as a control showed biofilm inhibition in the middle phase of *C. albicans* by 71.90% ± 0.96.

The effect of Masoyi oil on *C. albicans* membrane permeability was observed by monitoring the Propidium iodide influx (Figure 4). Propidium iodide is a fluorescent probe of DNA staining which can only enter the cell membrane if it is damaged or

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When the cell membrane was disrupted by the Masoyi oil, Propidium iodide could seep into the cytoplasm and bind into DNA. Through the inclusion of Propidium iodide, the Masoyi oil influenced the cells of \textit{C. albicans} by disrupting the cell membrane and then increase the permeability of the membrane.

The result of TEM analysis showed that \textit{C. albicans} experienced dramatic cell shape changes following the Masoyi oil application. The Masoyi oil increases the membrane cell permeability and causes the cytosol to leak out. Furthermore, the cell membrane of \textit{C. albicans} began to rupture (Figure 5 and Figure 6).

\textit{Kishimoto et al. (2005)} have proposed the antifungal effect of massoialactones is due to its effect on its respiratory system. Other related compounds showed a nonspecific, antifungal effect in which their binding to sulphydryl moiety and enzyme denaturation has led to membrane leakage (Pauli, 2006). Lactones have been reported to bind to sulphydryl moiety (Pratiwi et al., 2015).
CONCLUSION

The Masoyi bark essential oil containing the C-10 massoialactone as the major compound showed membrane disruption activity in C. albicans led to cell death. Farnesol as the quorum sensing compound in C. albicans was observed to be inhibited its production following sample application which may be due to insufficient cell population. This effect was also confirmed by no true hyphae or pseudohyphae formed following sample application.

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REFERENCES


