CHAPTER 1

INTRODUCTION

1.1 Background

Antimicrobial resistance is currently a very significant health concern that humans are facing due to the faster development of the resistance compared to the new therapeutic agent to solve it. Therefore, new therapeutic agents that possess slower or little impact on the development of antimicrobial resistance are needed. *Melaleuca alternifolia* (tea tree) essential oil possesses that desirable property. A single and multistep antibiotic resistance study revealed that exposure of tea tree oil has little effect to the development of resistance in tested strains of *S. epidermis, S. aureus,* and *E. coli* after (Katherine A. Hammer, Carson, & Rileya, 2012). In addition, no significant changes in antimicrobial susceptibility were found after repeated exposure to terpinen-4-ol, the main bioactive compound responsible for the antimicrobial activity of tea tree oil (TTO), as this substance is thought to induce insufficient adaptive measures to alter antimicrobial susceptibility. Aside from having a wide range of antimicrobial activity, TTO also possesses another excellent antimicrobial property, like the synergistic effect of TTO and other antimicrobial substances, including itraconazole, ciprofloxacin, gentamicin, cefixime, amphotericin B, fluconazole, and pristinamycin (Mertas et al., 2015).

Those properties of TTO make *M. alternifolia* an excellent antimicrobial agent. This powerful antimicrobial agent is mainly utilized as essential oil produced by steam distillation (C F Carson et al., 2006). However, prolonged contact with boiling water (hydrolysis, cyclization) in this method can cause artifacts and chemical alterations of terpenic molecules (Asbahani et al., 2015). Therefore, an altered profile of compounds compared with that of the original material may be reflected in the resulting isolate (Jajaei et al., 2010). Though it has been considered, no alternative extraction methods such as microwave assisted ethanol extraction has been utilized on a commercial scale (C F Carson et al., 2006).

TTO obtained by steam distillation also has several safety issues if administered orally. Although not necessarily directly correlated to human toxicity, values determined in animal studies indicate that TTO is orally toxic (K. A. Hammer, Carson, Riley, & Nielsen, 2006). Ingestion of TTO can be toxic as displayed by the LD_{50} in the rat, which is 1.9-2.6 ml/kg. Also, rats appeared lethargic and ataxic, and possess depressed activity levels 72 hours post dosing after given dose of ≤1.5g TTO/kg body weight. A clinical study with an oral solution of TTO also shows adverse effects happening in 66.7% patients with AIDS and fluconazole refractory oral candidiasis given alcohol-based TTO oral solution (C F Carson et al., 2006). These safety issues of TTO might cause the halt in the approval of TTO for the treatment of Dengue fever in Indonesia, AviMAC, by the local authorities since 2016 despite showing promising therapeutic effects to eliminate Dengue fever (Farmita, 2016; Ramadhan, 2016). Furthermore, encapsulation of essential oil to be consumed orally may cause an expensive production process, leading to a higher price of the end product compared to drugs obtained by other techniques such as maceration and soxhletation. Therefore, a safer and cheaper product of *M. alternifolia* is needed so that the health benefits of this plant can be felt by the communities.

The effect of other procedures to utilize *M. alternifolia* on the bioactive compound present and the antimicrobial activity is not extensively studied. Maceration and soxhletation are two of the simplest extraction method available in the development of a new therapeutic agent. They are also easy to be controlled and cheaper compared to encapsulation of essential oil, which may result in the more affordable cost of *M. alternifolia* end product that would be preferred by the people. The absence of heat in maceration makes this method suitable to extract thermolabile compounds, as oppose to both Soxhlet extraction and hydrodistillation. Soxhlet extraction allows a thorough extraction through a continuous cycle of condensed organic solvent contact with the sample (Gu et al., 2009). Furthermore, this method can sufficiently extract high molecular-mass compounds in addition to volatile compounds in some plants (Herzi, Bouajila, Camy, Romdhane, & Condoret, 2013; Wu, Wang, Liu, Zou, & Chen, 2015). The difference in compounds that could be extracted from these methods could greatly affect the antibacterial activity of *M. alternifolia* obtained. This study uses

Escherichia coli and *Staphylococcus aureus* to determine the antibacterial activity of *M. alternifolia* due to its ability to cause relevant health problems in Indonesia. This study aims to observe the different effect of *M. alternifolia* utilization, including soxhletation, maceration, and hydrodistillation to the compound obtained and the antibacterial activity. This study would hopefully allow better utilization of this powerful antimicrobial agent.

1.2 Research Objectives

This study aims to:

1. Detect various bioactive compounds obtained from maceration, soxhletation, and hydrodistillation of *M. alternifolia*.
2. Evaluate the antimicrobial activity of *M. alternifolia* obtained from maceration, soxhletation, and hydrodistillation.