

1. Introduction

1.1. Background

During the Covid-19 pandemic, people pay more attention to the COVID-19 itself as the growth and higher cases with mortality rate. But, in fact, besides the COVID-19, many other causes worsen the condition of the patient. One of them was Healthcare-Associated Infections (HCAIs). Nevertheless, the hospital plays the most prominent role in spreading HCAIs. Therefore, it was approved that this case required serious care in environment management (Irek et al., 2018). This statement is based on the fact that the US Center for Disease Control has identified more than 98,000 patients from 1.7 million hospitalized patients who died because of HCAIs (Haque et al., 2018). Amidst all of the HCAIs, the biggest challenges for the researcher are to find an adequate treatment of Gram-negative pathogens infection.

Furthermore, a study in 2018 from Baseti et al. stated that one of the Gram-negative bacteria that has a leading role in causing HCAIs was *Pseudomonas aeruginosa*. It can be found mostly in patients with immunocompromised conditions and critically ill patients. Not to mention, *P. aeruginosa* has caused a total of 51,000 healthcare infections per year (Lund-Palau et al., 2016).

The bacteria have been successful in colonizing in most types of environments. The reason behind it is the presence of the virulence factors of *P. aeruginosa* itself. It includes the production of the toxin, hemolysis protease, and biofilm formation. As already mentioned before, infection of *P. aeruginosa* is primarily because of the HCAIs. In this state, patients, clinicians, and people who work in the hospital were more prone to *P. aeruginosa* infection (Wilson & Pandey, 2020). For instance, breathing ventilator leads to ventilator-associated pneumonia (VAP), the use of a catheter, etc. (Ali et al., 2020; Jenny & Kingsbury, 2018). The previous research stated that nosocomial infection of *P.*

aeruginosa through ventilation reached 86%. In addition, it was also responsible for the high rate of mortality in the Intensive care unit (ICU). Moreover, 80-95% of CF patients experienced respiratory failure from this bacterial infection (Jenny & Kingsbury, 2018). Not to mention, this was because of necrotized *P. aeruginosa* pneumonia, contaminated respirators, and also localized infection. Despite this, bacteria can be isolated from several parts of the body including the skin, throat, stool, and gastrointestinal tract (Iglewski & Barbara, 1996). Therefore, an urge to develop a new treatment with fewer side effects is needed (Pang et al., 2018). As stated by WHO (World Health Organization), herbal medicine can be used as alternative medicine. Usually, the sources of herbal medicine are derived from plants that have many beneficial compounds inside them (Gupta & Kumar, 2017). For instance, *Camellia sinensis*, *Aloe vera*, and *Andrographis paniculata* have antibacterial properties that may prevent the growth of pathogens (Vasudeo & Sonika, 2015).

Aloe vera has been known worldwide as one of the plants with antibacterial, antifungal, and anti-inflammatory properties. This plant contains 98-99% water, and the rest are active compounds such as aloesin, aloin, aloe-emodin, saponin, flavonoids, amino acids, and vitamins aloe-mannan. Additionally, anthraquinones and their derivatives are found to have a substantial effect and potential as antimicrobial agents. Furthermore, the researcher believes *Aloe vera* can be an immune booster as it is an antioxidant (Jain et al., 2016).

Another plant that is already known for its antibacterial properties is *Camellia sinensis*. The component of polyphenols found in tea leaves was known as effective pharmacological properties against bacteria. When the polyphenol is oxidized, then it is called tannin. This compound has been identified as anti-carcinogenic, anti-inflammatory, and antibacterial effects against the pathogen (Vasudeo & Sonika, 2015). On the other side, sambiloto or *Andrographis paniculata* is an annual herb found in Sri Lanka, India. It is also widely used for medical purposes to reduce inflammation and fight viral and bacterial

infections. Since a long time ago, this plant is known in traditional Asian medicine as immune booster properties (Shalini & Narayana, 2015).

In the previous study, Utami&Andrayani (2020) conducted research to test the inhibition zone of *Aloe vera* against *P. aeruginosa*. However, this research was limited in the usage of the solvent. The solvent used in this experiment was only ethanol 96% mixed with *Aloe vera* extract. Therefore, it still lacks study about the effectiveness of using natural medicine against *P. aeruginosa*. Not so different from that, previous research has also conducted an experiment using *C. sinensis* extract. The ingredients were then diluted with several solvents, including water, ethanol, and chloroform but instead used a dry natural ingredient to make the extract (Sharma &Pundir, 2019). Meanwhile, there was less information in using *Andrographis paniculata* extract against *P. aeruginosa*. There was a study conducted by Alya& Laila (2020) using this extract with only ethanol as a solvent.

As the literature review, the author decided to choose these three plants as an experiment because of several reasons. It includes that all of the plants is easy to find in the daily life and accessible. Also the availability of these plants was still high. Another reason is that there is less study that using water as a solvent. It is also lead by anecdotal information that is spread in the society about these plants. Another reason was these plant contained polyphenol compound or secondary metabolites of polyphenol that is suspected as antibacterial properties against *P. aeruginosa*.

1.2. Objective

The objectives of this study are as follows.

- Testing the antimicrobial activity of *Camellia sinensis*, *Andrographis paniculata*, and *Aloe vera* to prevent the growth of *P. aeruginosa*. The reason behind choosing these three plants was because of improperly documented results from previous research.

- Investigating three different solvents, which are water, ethanol, and chloroform. The reason was that ethanol were able to dissolve polar substances such as phenolic compounds. Chloroform was used for separate the aqueous and organic compounds. Meanwhile, water was used to clarify the anecdotal information about natural remedies that exist in traditional society.
- Observing which one of the three ingredients may have the most significant impact in preventing *P. aeruginosa* growth.

1.3. Hypothesis

The author hypothesized that amongst all of the natural ingredients used as the treatment would result in successfully preventing the growth of *P. aeruginosa*. Thus, all solvents would successfully dissolve organic molecules contained in the natural ingredients. Among all of the extracts, *Aloe vera* was hypothesized to be the most effective against *P. aeruginosa*.