Chapter 1

Introduction

1.1 Background

Influenza is a highly pathogenic airborne virus that is transmitted through the aerosol of infected hosts. There are four types of influenza: A, B, C, and D. Influenza type A (IAV) is the most common Influenza emerging in the world and has caused several pandemics in the past (Boktor & Hafner, 2023). IAV lacks a proofreading function, thus it leads to a high mutation rate and produces new variants constantly. Currently, there are 18 subtypes of H (H1-H18) and 11 subtypes of N (N1-N11). The most prevalent subtype of IAV is H1N1; it is also the subtypes that cause seasonal influenza (Chauhan & Gordon, 2020). The major antigenic factors of IAV, hemagglutinin (HA) and neuraminidase (NA), are located on the outer surface of the viral envelope. Matrix protein, the most abundant and conserved protein across many IAV subtypes, forms a layer underneath the viral membrane. M protein is the most abundant protein of IAV and is conserved in many subtypes of IAV. It makes the M protein become the most used and suitable primer for IAV viral quantification. The M gene expression in IAV is directly related to the viral number quantified. Thus, if the M gene expression is downregulated, it indicates a decrease of IAV number (Peukes et al., 2021). To treat IAV, there are four antiviral drugs recommended by the CDC that include oseltamivir, zanamivir, peramivir, and baloxavir marboxil. Oseltamivir, peramivir and zanamivir target the NA protein, which is one of the major antigenic factors of IAV. The inhibition of NA will cause no virion release as NA role is to cleave sialic acid to release the virion from infected cells (Gao et al., 2024). Unlike the other three antiviral drugs, baloxavir marboxil works by inhibiting PA protein, a crucial protein for viral transcription (Tejus et al., 2022). Despite the effectiveness of the antiviral drugs, the risk of antiviral drug resistance is increasing. The resistance most likely occurred on wild-type infection and caused the drugs to be ineffective against IAV (Smyk et al., 2022). Thus, in this case, herbal medication could be an alternative to IAV antivirals. In Indonesia, herbs are widely used for traditional medication practice as herbal medication has also been part of the culture for a long time. There are many

Indonesians who still choose to consume herbal medicine for common ailments, including influenza (Kristianto et al., 2022). The other advantage of herbal medication is that it could offer a cheaper price and wider distribution than drugs sold at pharmacies.

Indonesia has a wide variety of plants and herbs due to its tropical climate, which is suitable for most of the plant species' growth. Itchy Leaves with the Latin name *Laportea decumana*, is a herbal plant that is endemic to Papua Island. It is a herbal that has been used by local people living in Papua for traditional medicine. It has an analgesic effect and has been used as the treatment for muscle fatigue or muscle ache, and also as a pain reliever (Basy et al., 2024). There are several active compounds contained inside the itchy leaves. From the ethanolic extract of *L. decumana*, the compounds isolated are alkaloids, terpenoids, triterpenoids, saponins, and tannins. Due to the bioactive compounds contained, *L. decumana is also* being utilised to become medications of some diseases aside from just treating the muscle aches and becoming a pain reliever (Siahaya et al., 2025). The whole extract of *L. decumana* has only ever been researched as the NDV antiviral; thus, its effect and the mechanism of action of how it performs the antiviral activity have not been thoroughly investigated (Tee et al., 2024). Even though the antiviral activity of *L. decumana* has never been researched, there is a high probability of it becoming antiviral due to the active compounds contained in it. Moreover, through the *in silico* study, the preliminary study showed that *L. decumana* compounds are able to strongly bind to the protein of IAV and potentially inhibit it. The *in silico* analysis has been conducted before; results suggest that *L. decumana* compounds have similar interactions and binding sites with the known inhibitor drug, which is zanamivir, predicting the high possibility of protein inhibition activity by the compounds. Depending on the solvent used in the maceration process, the active compounds contained in the *L. decumana* extract may also differ. Despite having five active compounds, there are only three active compounds detected in the methanolic *L. decumana* extract according to the unpublished data of preliminary study. The methanol extraction of *L. decumana* is important for this study because it could extract the main compounds needed, which are triterpenoids, terpenoids, and

alkaloids. From the previous preliminary *in silico* study, triterpenoid compounds have the most negative binding affinity, which indicates the strongest binding (-7.5 to -8 kcal/mol toward NA and -4.7 to -5.4 kcal/mol toward HA) amongst all compounds tested. For terpenoids and alkaloids, they are the most documented active compounds with antiviral activity towards IAV (Guo et al., 2022).

1.2 Objective

- To fractionate *L. decumana* methanol extract into three distinct compound-enriched fractions (triterpenoid-enriched, terpenoid-enriched, and alkaloid-enriched fractions) using column chromatography.
- To assess the antiviral activity of *L. decumana* fractionated extracts by measuring M gene expression using qRT-PCR.

1.3 Hypothesis

- The *L. decumana* methanolic extract will be fractionated into triterpenoid-enriched, terpenoid-enriched, and alkaloid-enriched fractions.
- The fractionated extracts of *L. decumana* will exhibit antiviral activity, indicated by the downregulation of M gene expression that will be measured by qRT-PCR analysis.