Chapter 1

Introduction

1.1 Background

The Coronavirus Infectious Disease 2019 (COVID-19) pandemic is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As of the 7th of June 2023, the virus has led to more than 767 million positive cases and over 6.9 million deaths globally, while positive COVID-19 infections in Indonesia have reached over 6.8 million people with over 161 thousand deaths (World Health Organization, 2023). While vaccines have successfully paved their way for effective protection against COVID-19, several pharmacological strategies have also been proposed for COVID-19 prevention and treatment (Cascella et al., 2022). Among them are the administration of interferon (IFN), interleukin 6 (IL-6) receptor antagonists, chloroquine, and hydroxychloroquine, which exert several antiviral mechanisms including endocytosis-mediated blocking of viral entry, endosomal acidification inhibition, glycosylation interference on angiotensin converting enzyme 2 (ACE2), RNA synthesis inhibition from nucleotide analogue and nucleoside, as well as protease inhibition (Frediansyah et al., 2021). Out of all the mentioned strategies, Remdesivir is the only nucleotide analogue prodrug indicated for COVID-19 with compliance to United States Food and Drug Administration (USFDA) guidelines and standards, which acts by interfering with the nsp12 polymerase (Zhang & Zhou, 2020). Despite its positive antiviral response against SARS-CoV-2, Remdesivir has limited clinical effectiveness in the early clinical trials (Young et al., 2021) and does not exert significant reductions in COVID-19 mortality rate (Tasavon Gholamhoseini et al., 2021).

Two-thirds of the viral genome of SARS-CoV-2 are composed of non-structural proteins (NSPs) which are fundamental for viral replication and protein maturation (Rohaim et al., 2021). The SARS-CoV-2 life cycle is initiated with the proteolytic cleavage of the non-structural 3-chymotrypsin-like protease ($3CL^{pro}$) alongside papain-like proteases (PL^{pro}). With the former having a higher amount of cleavage

sites, $3CL^{pro}$ undergoes autocatalytic cleavage by the polyprotein. This can then be processed by several other non-structural proteins to undergo further viral replication, thereby increasing the generation of new viral particles, as well as the spread of infection (Kuzikov et al., 2021). Due to its central contribution in the effects as mentioned above, $3CL^{pro}$ can therefore be considered as a promising drug target (He et al., 2020). A marketed antiviral medication which targets SARS-CoV-2 $3CL^{pro}$ is Paxlovid™, composed of two antiviral drugs; nirmatrelvir and ritonavir. While multiple clinical studies have reported the high safety and efficacy of Paxlovid in reducing the risk of COVID-19 mortality and severity of symptoms (Liu et al., 2023; Najjar-Debbiny et al., 2023; Zheng et al., 2023), it can only be authorized for emergency use in moderate-to-high severity, as well as hospitalized patients (Pfizer, 2023). The search of $3CL^{pro}$ inhibitors from herbal plants can therefore be a more affordable and readily accessible option.

A study conducted by Jo et al. (2020) created a database of flavonoids to investigate compounds with inhibitory activity on SARS-CoV using the Förster resonance energy transfer (FRET) method. Its implementation allowed the detection of a number of flavonoids with various inhibitory levels. This study demonstrated the integration of biochemical tests and docking predictions and its usefulness to develop more inhibitory flavonoid derivatives of various flavonoid scaffolds. Understanding the high conservation of $3CL^{pro}$ gene sequence in SARS-CoV and SARS-CoV-2, flavonoids are expected to also be able to potentially exert inhibitory activity against the latter form of the virus.

Research conducted by Huang et al. (2020) in China investigated traditional chinese medicine as a form of intervention therapy, which could work against COVID-19. A clinically significant efficacy is achieved, but the underlying pharmacological mechanism remains unclear. The research demonstrated that proteins ACE2 and $3CL^{pro}$ directly contributed to the life cycle and subsequent infection of SARS-CoV-2 to the host cell. The protein markers can be used as drug targets to reduce or stop the severity of SARS-CoV-2. The results from Huang et al. (2020) demonstrated that several

flavonoid bioactive compounds including quercetin, luteolin, kaempferol, and isorhamnetin are among the main candidates found in Chinese medicines for COVID-19 management. ACE2 and $3CL^{pro}$ are targeted through an inhibitory mechanism, whereby inflammatory mediators are inhibited, free radicals are eliminated, and immunity is eventually regulated and restored.

Exploration of potential bioactive compounds from herbal resources have become more imminent following the increased supply and demand of complementary and alternative medicines (CAM) for the prevention, symptom/s relief, and/or as a supplementary treatment amidst the COVID-19 pandemic (Paudyal et al., 2022). In particular, several flavonoid compounds found in fennel seeds have been reported to have promising $3CL^{pro}$ inhibitory effects. Fennel seed or *Foeniculum vulgare semen* is native to Southern Europe and several parts of Asia, including Indonesia (Britannica, 2022). The seeds are commonly employed for its wide variety of pharmacological effects, with antiviral included (Badgujar et al., 2014), as well as for its concentrated load of bioactive compounds, with one of them being flavonoid compounds (Malin et al., 2022). Particularly, kaempferol and quercetin derivatives present in *F. vulgare* demonstrated a high docking score, thus illustrating its good binding affinity and subsequent inhibition with the active site of SARS-CoV-2 $3CL^{pro}$ (Mouffouk et al., 2021).

F. vulgare is one of the vast amounts of flora which are abundantly grown and readily found in Indonesia, and is also known to naturally contain flavonoids as its major class of secondary metabolites (Badgujar et al., 2014). Furthermore, there are also currently no previous studies as of the period this thesis was created, which investigated the types of flavonoid compounds present (e.g. quercetin, kaempferol, and its derivatives) in *F. vulgare* seeds and its level of inhibition against SARS-CoV-2 $3CL^{pro}$. This can ultimately aid in supporting governmental efforts to accelerate healthcare research and innovation toward the prevention and reduction COVID-19 cases (Putera et al., 2022).

1.2 Objective

The aim of this research is to screen several flavonoid bioactive compounds, such as quercetin, kaempferol, and its derivatives present in *F. vulgare* seed Extract and assess its potential inhibitory effects against SARS-CoV-2 $3CL^{pro}$.

1.3 Hypothesis

F. vulgare seed is expected to contain flavonoid compounds which could exert inhibitory activity against SARS-CoV-2 $3CL^{pro}$, and hence could potentially act as an antiviral CAM candidate for further COVID-19 drug screening.