# Chapter 1: Introduction

## 1.1. Background

*Clostridium difficile* infection (CDI) is a healthcare-associated infection caused by *Clostridium difficile* (*C. difficile*), an anaerobic spore-forming gram-positive bacterium (Czepiel et al., 2019; Ngamskulrungroj et al., 2015;). Long-term exposure of antimicrobials is the main factor that allows the proliferation of *C. difficile* in the intestine through a change of gut microflora (Collins & Riley, 2019; Harnvoravongchai et al., 2018; Peng et al., 2017). The symptoms of CDI vary from mild (e.g., antibiotic-associated diarrhea) to severe diarrhea (e.g., pseudomembranous colitis) and is caused by Toxin A and B, which are produced by *C. difficile.* These toxins may destroy the tight junctions of intestinal epithelial cells (Banawas, 2018; Harnvoravongchai et al., 2018).

Currently, metronidazole and vancomycin are the first-line treatment for CDI (Banawas, 2018; Peng et al., 2017). However, according to Goudarzi et al. in 2014, *C. difficile* has been reported to be resistant against metronidazole and vancomycin based on the Clinical and Laboratory Standard Institute (CLSI), which is an institute that assigns standard and guidelines to professionals in the medical field (Weinstein & Lewis, 2020). High background antibiotic-resistant and genetic versatility allow *C. difficile* to develop defensive mechanisms against these antibiotics (Harnvoravongchai et al., 2018). Although there are several alternative treatments such as fecal transplantation and phage therapy to treat CDI, limitation in the immunological side (e.g., excessive immune stimulation) is one of the significant concerns (Harnvoravongchai et al., 2018; Zeng et al., 2019).

One of the sources of the novel therapeutic options mostly come from herbs and natural products. Asiatic acid (AA) is one of the natural products that have pentacyclic terpenoid that is mainly found in *Centella asiatica* (traditional medicine herb) (Lv, Sharma, Zhang, Wu, & Ding, 2018). It has been shown to exhibit antimicrobial activity, anticancer, and neuroprotective activities (Harnvoravongchai et al., 2018; Lv, Sharma, Zhang, Wu, & Ding, 2018). According to Harnvoravongchai

et al., 2018, AA can inhibit *C. difficile* within a 10-20 µg/ml concentration range. In order to increase and broaden AA's antimicrobial activity, semi-synthetic AA or AA derivatives can be generated by chemical modification to its structure (Rajeev, 2016).

In this study, modified AA, namely: AT1, LS1, and LS2 acquired from the Department of Chemistry Mahidol University Faculty of Science, was used to investigate the antimicrobial activity against *C. difficile* 630 and R20291. Those strain were used in this study because of several factors such as 1) those strains commonly cause CDI around the world which showed that *C. difficile* R20291 cause the epidemic in the health care system and *C. difficile* strain 630 lead into endemic in health care system (Stabler et al., 2009; Stabler et al., 2010; Valiente et al., 2015); 2) both of *C. difficile* 630 and R20291 has been studying very well and already has the complete genome sequence; 3) most of the *C. difficile* strains resistance against clindamycin, meanwhile, these *C. difficile* strain have multiple-drug resistance against several antibiotics, such *C. difficile* strain 630 has resistance against tetracycline, meanwhile, the R20291 in fluoroquinolones (Stabler et al., 2009; Stabler et al., 2010; Valiente et al., 2015); 4) *C. difficile* R20291 known to lead more severe diarrhea which can cause higher mortality and more recurrences compared to *C. difficile* 630; and 5) there are 234 more additional genes in *C. difficile* R20291 compared to *C. difficile* 630 which lead into the antibiotic resistance, toxicity, and in phenotyping difference (Stabler et al., 2009; Stabler et al., 2010; Valiente et al., 2015).

## 1.2. Objective

This study was conducted to investigate the antimicrobial activity of asiatic acid derivatives against *C. difficile* 630 and R20291 using minimum inhibitory concentration (MIC) and time-kill kinetic assay.