## CHAPTER 1: INTRODUCTION

Malaria is a fatal parasitic infection spread to humans by female Anopheles mosquitoes carrying Plasmodium parasites (WHO, 2021a). Statistically, malaria affected 229 million individuals worldwide in 2019, according to the World Health Organization (WHO) (WHO, 2021a). South-East Asia is the second most globally impacted by malaria, with more than 200,000 cases in Indonesia (WHO, 2021a; Kemenkes RI., 2020; WHO, 2021b). Additionally, it is easier for the highly temperature-sensitive Anopheles mosquito and Plasmodium parasite to spread malaria due to Indonesia's ideally steady climate of moderately high humidity and heavy rainfall season. This favorable climate further promotes adult Anopheles mosquitoes' survival and adds more breeding sites for population growth (Abiodun et al., 2016; Hasyim et al., 2018; Weaver, 2014; Yamana & Eltahir, 2013). Regardless, among the five known human-infecting Plasmodium parasite species, *Plasmodium falciparum* gave rise to the most dreadful malaria form in humans and accounted for half of the malaria cases in 2018 (WHO, 2021b; Zekar & Sharman, 2020). In addition, *P. falciparum* is easily cultured in the laboratory; thus, numerous studies have revolved around this parasite compared to the other Plasmodium parasites.

Presently, drug therapies to treat malaria would provide a certain amount of success; However, some factors, like side effects and antimalarial drug resistance, still require consideration. Not to mention, the parasite resistance emergence to most antimalarial drugs such as the first-line artemisinin, chloroquine, piperaquine, atovaquone, and others (Phyo & Nosten, 2018). Most antimalarial drugs are schizonticidal blood drugs that target the parasite's asexual blood stages. Other antimalarial drugs such as gametocytocidal or tissue schizont respectively target the sexual blood stages and the parasite’s dormant stage in the liver (Shibeshi et al., 2020).

In contrast to other infectious diseases, the malaria parasite is competent at resisting the precise cellular drug targets by mutating the targets and non-specific efflux of the drugs (Shibeshi et al., 2020). For instance, antifolate drugs such as sulfadoxine and pyrimethamine respectively inhibit dihydropteroate synthetase and dihydrofolate reductase, thus reducing the synthesis of folate and

DNA for the parasite. Parasites resistant to these antifolates would specifically mutate both dihydropteroate synthetase and dihydrofolate reductase (Shibeshi et al., 2020). Another example is artemisinin, which alkylates heme proteins and lipids to generate free radicals that are toxic to malaria parasites. For a real-life example of *P. falciparum* resistance, its resistance to artemisinin-based combination therapy, 4-aminoquinoline drugs such as chloroquine, and antifolate drugs are presented in various regions of Indonesia due to *P. falciparum* mutating precisely at drug targets (Basuki et al., 2018; Lubis et al., 2020; Ratcliff et al., 2007; Reteng et al., 2017; Syafruddin et al., 2005). Nevertheless, the geographic distribution of the resistant *P. falciparum* strain indicates that other countries or regions in that same country may not present the same resistance (Phyo & Nosten, 2018; Syafruddin et al., 2005).

Other factors that aid in the persistence of malaria infection would be the mosquito vector evolving to be insecticide-resistant and the absence of an available malaria vaccine (Ellis et al., 2010; Hamid et al., 2017; Osier et al., 2014; Sumarnrote et al., 2017). As of now, many of the blood-stage vaccine candidates for malaria showed strain specificity but were limited in terms of efficacy and protection against clinical outcomes (Ellis et al., 2010). Therefore, as mentioned before, the currently used antimalarial drugs mainly target the parasite blood stage. Symptoms of the *P. falciparum* infection originate from its asexual blood stage, which develops inside the red blood cells (RBCs), whereas its sexual blood stage is vital for parasitic development inside the human host and mosquito vector for transmission (Buffet et al., 2011; Venugopal et al., 2020). Therefore, it is a top priority to identify and validate numerous novel druggable targets, especially those expressed at multiple blood stages of the parasitic growth in the host. This way, we will have numerous backup drug targets in case *P. falciparum* alters or mutates some of the drug targets once again.

Hence, this is mainly why numerous studies on traditional Chinese medicinal herbs as their natural products display notable pharmacological activity, reduced toxicity, and little to no side effects for use as novel treatments (Czechowski et al., 2019; Yin et al., 2018). The principal antimalarial drugs, artemisinin and chloroquine, came from herbal medicines (Oladeji et al., 2020).

Thus, it is possible to successfully treat malaria using herbal plants, as proven in ethnobotanical surveys of Indonesia and worldwide (Abdillah et al., 2017; Al-Adhroey et al., 2010; Oladeji et al., 2020).

*Gynura divaricata* (Lour.) Merr. (common name: fairy grass or *daun dewa*) is a type of traditional Chinese medicinal herb known to be effective in treating various human-related diseases, ranging from toothaches, sore eyes, pulmonary tuberculosis (Chen et al., 2009; Xu & Zhang, 2017), corns (also termed as clavus) (Moeloek, 2017), to insulin-resistant type 2 diabetes (Li et al., 2018; Xu & Zhang, 2017; Yin et al., 2018). It belongs to the Asteraceae family and, other than in South China, is also found in the Sumatra region of Indonesia (Moeloek, 2017; Vanijajiva & Kadereit, 2011). A reason to explore the antimalarial potential of *G. divaricata* is that the leaf portion of the *G. divaricata* sister plant, *Gynura procumbens (Lour.) Merr.*, demonstrates an antimalarial ability by inhibiting malaria parasite growth and prolonging the survivability of *P. falciparum*-infected mice (Ashraf, 2019; Tan et al., 2016; Vejanan et al. 2012). However, there have been no studies focusing on the antimalarial activity of *G. divaricata*.

Furthermore, the root, stem, and leaf parts of *G. divaricata* are edible and predominantly filled with flavonoids, phenolic acids, alkaloids, polysaccharides, and others (Xu & Zhang, 2017). Flavonoids are known for their various properties, including being anticancer (Cibin et al., 2010; Fang et al., 2009), antibacterial (Kouam et al., 2007; Ismat et al., 2010), antioxidants (Gould & Lister, 2006; Heim et al., 2002) and antimalarial (Rudrapal & Chetia, 2017). As *G. divaricata* has abundant flavonoids, it has a conspicuous aptitude for natural antioxidant activity (Xu & Zhang, 2017). Due to the flavonoids' complex synergistic or antagonistic nature, the *G. divaricata* flavonoids receiving proper experimental design and required concentrations will inhibit parasite growth and protect hosts with chronic malarial infection (Delhaye et al., 2018; Schroeter et al., 2002; Wezena et al., 2017).

Hence, we aim to investigate the antimalarial activities of the *G. divaricata* compounds against several asexual and sexual blood-stage drug targets expressed in *P. falciparum* via *in-silico*

studies. The reason is that *in-silico* studies are generally low-cost and aid in directing and validating *in-vivo* and *in-vitro* studies. The selected protein targets are either or both of the asexual and sexual blood stages expressed in various pathways related to invading the RBCs, parasitic growth or development, host plus vector immune evasion, and parasite transmission to the mosquito vector. Afterward, the resultant inhibited blood-stage drug targets by *G. divaricata* compounds may prove *G. divaricata* to be a potential antimalarial drug.