Chapter 1

Introduction

1.1 Background

Malaria is one of the most prominent and deadly parasitic diseases worldwide spread through the bites of infected female mosquitoes from the genus *Anopheles* (WHO, 2017). It is one of the most widespread diseases that has become endemic in about 100 developing countries. These diseases are known to kill an estimated 1.2 million people each year in Africa and in 2019 alone, it affected 229 million people and killed 409,000 people (WHO, 2001; WHO, 2020). Among the plasmodium species, *Plasmodium falciparum* is known to be the most fatal and deadliest malaria parasite responsible for the majority of the death cases. Moreover due to the increase of resistance to the current antimalarial drug as well as the high mutation rate of this parasite, it becomes even harder to eradicate and control this disease (Burkard et.al., 2016).

Antimalarial drugs can either be schizonticidal blood drugs which target the parasite's asexual blood stage, gametocytocidal which target the sexual blood stage or tissue schizont that target the parasite's dormant stage in liver (Shibeshi et.al., 2020). Although the current drug therapies are still useful and provide a certain amount of success, there is a deliberation of usage to the current antimalarial drug. This is due to the increase of resistance especially to most of the first-line drugs of choice including artemisinin and chloroquine as well as the capability of malaria parasites to mutate the drug target hence forming more resistance (Phyo & Nosten, 2018). Therefore, it becomes highly significant to search and identify more novel treatment agents to control this disease.

The use of medicinal plants as the treatment for malaria has been carried out for thousands of years. Plant-derived compounds such as quinine and artemisinin are isolated from Cinchona bark and Chinese plant *Artemisia annua* has been used as the main herbal medicine to treat malaria (Titanji

et.al., 2008). This herbal plant also has been proven to successfully act as antimalarial agent against chloroquine-resistant *P. falciparum* strains (Schwikkard & Van Heerden, 2006). In addition to the resistance of parasitic strains, including *P. falciparum,* to the current modern drug available, there is a tendency and preference of herbal plants usage as treatment medicine, which becomes the booster behind the interest of uncovering further possible alternatives for more efficacious antimalarial drugs.

One of Indonesia's native medicinal plants is *Gynura divaricata* is also known as *Daun Dewa, known* to be effective in treating various human-related diseases which belong to the Asteraceae family (Chen et.al., 2009). Based on the previous study (unpublished), the herbal plant *Gynura divaricata* was observed to inhibit growth of *P. falciparum* with an IC50 of 0.692 mg/mL. *In-silico* research conducted by Widjaja (2022) using molecular docking also reported a strong interaction between PfCDPK1 protein, one of drug targets in malaria, with β-sitosterol compound found in *Gynura divaricata*. This shows a strong correlation which requires further evidence through *in-vitro* research validating β-sitosterol is responsible for the antimalarial properties exerted by *Gynura divaricata*.

Administration of xenobiotics such as antimalarials can increase the overproduction of reactive oxygen species (ROS) which may attack lipid resulting in the formation of lipid peroxide producing malondialdehyde (MDA) as one of its oxidation product (Santo et.al., 2016; Deavall et.al., 2012; Sampson et.al., 2019). Therefore, the assessment MDA concentration in the presence of antimalarial agents is commonly executed to evaluate its activity through the occurrence of oxidative damage by ROS. Hence, this study aims to validate the antimalarial properties of *Gynura divaricata* through investigating the antimalarial activity of β-sitosterol obtained from the extract.

1.2 Objective

In this research, the aim is to isolate β-sitosterol from the *Gynura divaricata* leaf extract and validate the antimalarial activities of *Gynura divaricata* through the action of concentrated β-sitosterol on *P. falciparum* as well as elucidate its mechanism of action against *P. falciparum*.

The specific objectives for this research are as follows:

- To obtain β-sitosterol rich fraction from *Gynura divaricata* leaf extract.
- To test the 50% inhibitory concentration (IC50) of β-sitosterol from *Gynura divaricata* against *P. falciparum*.
- To investigate the oxidative damage to *P. falciparum* upon treatment with concentrated β-sitosterol from *Gynura divaricata* through reactive oxygen species (ROS) assay.
- To investigate the mechanism of β-sitosterol as antimalarial through DNA fragmentation assay.

1.3 Hypothesis

The hypothesis of this research is that the antimalarial activity from the *Gynura divaricata* extract comes from the compound β-sitosterol which would exert oxidative damage in the parasite and induce parasite death by apoptosis.