Chapter 1

Introduction

1.1 Background

Plasmodium falciparum, a kind of single-celled parasite, is the main cause of malaria and continues to have a major impact on public health worldwide, especially in areas with limited access to healthcare. A significant obstacle in the effort to eradicate malaria is the rise and spread of drug-resistant forms of *P. falciparum*. These specific strains of malaria parasites have been discovered to reduce the effectiveness of antimalarial drugs. To personalize treatment plans and put into place containment procedures that are effective, it is very necessary to detect these medication-resistant strains as quickly and correctly as possible (Zekar & Sharman, 2022).

Malaria is prevalent in some locations, particularly rural areas where access to advanced laboratories capable of detecting resistant *Plasmodium falciparum* strains is sometimes limited. Malaria poses a considerable public health risk in these locations due to a lack of effective diagnostic techniques and the presence of drug-resistant parasites (Omondi et al., 2023). The rural location can make successful malaria care difficult since patients may not have access to the essential medical facilities or skilled specialists to provide accurate diagnoses and treatments. This can result in poor treatment results and greater death rates, thus, it is critical to establish measures to address these issues and enhance access to healthcare services in these locations (Kayiba et al., 2024).

This study addresses the important problem of detecting drug-resistant strains of *P. falciparum* by providing innovative techniques of isothermal amplification that were particularly developed for this purpose. Loop-mediated isothermal amplification (LAMP) offers a practical option for promptly detecting drug resistance, taking advantage of its beneficial characteristics (Lau et al., 2016). The application of isothermal amplification techniques offers numerous benefits for adoption in settings

with limited resources, owing to their ease of use, expedited amplification process, and reliable performance. These are the types of locations in which the incidence of malaria is frequently elevated.

Genomic and proteomic study reveals that drug-resistant *P. falciparum* is linked to crucial regulatory genes. The PfMDR1 locus is linked to chloroquine resistance (Al-Mekhlafi et al., 2022). The development of resistance to artemisinin-based combination treatment (ACT) is attributed to genetic variations in the pfkelch13 locus. Through the identification of these genetic variations, drug-resistant strains of *P. falciparum* can be detected (Nsanzabana, 2019).

This project's objective involves advancing and enhancing isothermal amplification assays, primarily focusing on optimizing LAMP to amplify loci or genes linked to drug resistance. The assays are then compared thoroughly with PCR in detecting drug-resistant strains, especially when parasitemia levels are relatively low.

1.2 Objective

The objective of this study is to develop a method for amplifying *P. falciparum* genes associated with drug resistance using an isothermal application methodology. The results of the tests are going to be compared to PCR.

1.3 Hypothesis

Compared to PCR, LAMP amplifies DNA in a shorter time and at a constant temperature, allowing it to be done with fewer resources. LAMP uses numerous primers (usually six) that each recognize different sections of the target sequence, resulting in similar gel electrophoresis results with PCR.