The Potential of Allicin, Ajoene and Quercetin Contained in Garlic (*Allium sativum*) as a Promising Anti-Malarial

Bagy RG¹, Prakoso D¹, Dewi RS¹, Kantohe T¹, Tuda JSB²

1 IMedical Student, Faculty of Medicine, Sam Ratulangi University, North Sulawesi, Indonesia
2 Division of Clinical Parasitology, Faculty of Medicine, Sam Ratulangi University, North Sulawesi, Indonesia

Abstract
Malaria is an infectious disease with a high morbidity and mortality rate caused by Plasmodium and is transmitted through the Anopheles mosquito. Garlic (*Allium sativum*) has active substances that can be used as medicinal ingredients. Several active substances, namely allicin, ajoene, and quercetin contained in garlic have the potential to act as an effective and safe anti-plasmodium for the host. This writing is a literature review using relevant literature. The results of the study concluded that the active ingredients in garlic have potential to be a new antimalarial agent, and it is hoped that it can be a cheaper and more effective malaria treatment so that drug resistance and morbidity and mortality from malaria can be reduced.
1. Introduction

Malaria is an infectious disease caused by Protozoa belonging to Apicomplexa, namely Plasmodium which is transmitted by Anopheles mosquito. Plasmodium that can cause malaria consists of P. falciparum, P. vivax, P. ovale, and P. malariae. Malaria is causing high morbidity and mortality worldwide. Globally in 2016, there were estimated 216 million cases of malaria causing 445,000 deaths. This is certainly a health concern for various countries, especially in endemic areas. In 2016, USD 2.7 billion was allocated by countries with malaria endemic and international partners in efforts to prevent and eradicate malaria.\textsuperscript{1,2}

The diagnosis of malaria can be established by clinical symptoms in the form of the classic triad of malaria symptoms, consisting of fever, chills and sweating and the presence of other accompanying symptoms such as headache, nausea, and vomiting. A definite diagnosis of malaria is established through microscopic examination by finding plasmodium in thick or thin peripheral blood smear. Another technique that can be used is the examination of malaria antigens with the immunochromatographic test technique, to find Deoxyribonucleic acid/Ribonucleic acid by Polymerase Chain Reaction.\textsuperscript{3,4}

Since 2004, Artemisinin Combination Therapy has been started in the form of artemisinin amodiaquine to replace chloroquine preparation that has been resistant, but in less than 2 years, this combination has been reported a decrease in the efficacy so that it is replaced with another combination, namely dihydroartemisinin-piperaquine (DHP) which is considered more effective.\textsuperscript{5}

Garlic has more than 2000 active substances such as volatile, water-soluble and oil-soluble organosulfur components, dietary fiber, flavonoids, pectin, quercetin along with other sulfur components such as alliin. Natural degradation of alliin can produce other useful active substances in the form of thiosulfinate (allicin), ajoene, vinylthiin, sulfides (Diallyl disulfide, diallyl trisulfide). Of the various active ingredients that have been identified, ajoene, allicin, and quercetin are believed to be some of the active substances that are useful as antimalarials.\textsuperscript{6-8}

Efforts in the discovery and use of cheap and effective new sources of drugs are essential to prevent rapid resistance and treat malaria so that malaria morbidity and mortality can be reduced.

2. Discussion

2.1 Garlic (Allium sativum)

Garlic (English), \textit{Allium sativum} (Latin) is a plant that is in the shape of a bulb, widely distributed throughout the world, and grows well in dry and acidic soil, with sufficient light. Garlic has hermaphrodite flowers and is pollinated by insects. \textit{Allium sativum} comes from the \textit{Alliaceae} family, and is now cultivated throughout the world so it is easy to find in various places.\textsuperscript{9,10}

Garlic is believed to have come from Central Asia, in Western China, around Mount Tien Shan to Kazakhstan and Kyrgyzstan. Garlic can be consumed as a raw vegetable, or it can be processed in the form of oil, extract, or powder. Since 2600 – 2100 BC, the Sumerians used garlic for health purposes, which initially developed in China, then spread to Japan, Korea and the rest of the world. Garlic has various medicinal benefits, including as an anticancer, anti-inflammatory, antibacterial, antioxidant and is beneficial for the immune and cardiovascular systems.\textsuperscript{6-8} It is not surprising that in various parts of the world garlic has various medical terms used today such as 'Russian Penicillin', 'natural antibiotic', 'plant viagra', 'amulet plant', 'snake grass', and so on.\textsuperscript{5,11}

2.2 Ajoene

Ajoene (4,5,9-trithiadodeca-1,6,11-triene 9-oxide) is a sulfur-containing compound derived from \textit{Allium sativum} using ethanol extraction.\textsuperscript{12,13} Ajoene is formed from alliin and converted from alliin by an enzymatic process. Several studies have shown various functions of ajoene, including as antifungal,\textsuperscript{14,15} antithrombotic,\textsuperscript{16,17}
antiviral,18 antitumor,19,20 and even antiparasitic.12 One of the studies conducted in 1994 by Perez et al., showed that allyl sulfide compounds, the fat-soluble in ajoene, can reduce the degree of parasitemia of Plasmodium berghei infection in rats. Trials also showed that a single oral dose of ajoene of 50 mg/kg can stop the development of parasitemia at the time of infection without the appearance of signs of acute toxicity due to the active ingredient. The combination of ajoene and chloroquine can also prevent the further development of parasitemia in infected mice.12 There is no further studies on the antiparasitic effect of ajoene on other anthropophilic Plasmodium have been carried out in clinical trials.

Figure 1. Chemical Structure of Ajoene (4,5,9-trithiadodeca-1,6,11-triene 9-oxide) 6

2.3 Allicin

Allicin is a thiosulfinate sulfur-containing compound which is most abundant in garlic. Allicin is formed when alliin reacts with the enzyme alliinase.21 Alliin and alliinase are located in different parts of garlic, so garlic needs to be ground first to obtain allicin.21 Garlic has long been known to have an antimicrobial function, and there are many documentations of this function, one of which is by Louis Pasteur.21–24 In Malaria parasites, allicin acts in the pre-erythrocyte phase and has also been shown have a role as a prophylactic and enhance the host immune system.21,23–25

In the pre-erythrocyte phase, allicin works by inhibiting the cutting of the circumsporozoite protein (CSP) of the Plasmodium parasite. Plasmodium is enveloped by CSP as cell wall adhesion, at the time of invasion of plasmodium into cells, the CSP needs to be cut by cysteine protease derived from parasites.21,23 Allicin acts by reacting the free sulfhydryl group on the active site of the cysteine protease.21 The thiol-sulfide exchange reaction causes inhibition of the active site of cysteine protease.26 A study using pure allicin with a cysteine inhibitor protease E-64 as a control found that the inhibitory effect of allicin at 10, 25, and 50 M was equivalent to that of 10 M E-64.21 Another study using garlic oil in monotherapy of Plasmodium berghei showed an increase in the length of survival of mice compared to the control group (~8 days for intervention and ~4-6 days for control).23 To rule out toxicity mechanism against plasmodium, a study with propidium iodide staining comparing allicin with a control group had showed there was no significant difference in cells stained by propidium iodide between the two groups.21

Allicin has the potential to act as a prophylactic through two mechanisms, preventing the invasion of the Plasmodium parasite and increasing the body's immune system.21,23–25 Another study also showed that sporozoites treated with allicin reduced the invasion according to the dose (37% for 10uM and 89% for 50uM), the results were similar to the control group E-64, although administration of allicin to host cells did not show any signs of significant results.21 Allicin has also been shown to increase immune response and protect against malaria infection by significantly increasing Interferon Gamma (IFN-γ), Tumor Necrosis Factor (TNF), and Nitric Oxide (NO) as well as promoting dendritic cell maturation.24 In a lesser degree, allicin also increased Interleukin 12P70 (IL-12P70) and Cluster Differentiation 4+ (CD4+) expansion. Large doses of allicin can increase levels of regulatory T cells, but do not show a significant increase in Interleukin 10 (IL-10). Thus, allicin did not have a significant immune system inhibitory effect.24 Another study also showed there was an increase in Immunoglobulin G (IgG) in the group of rats that received arteether plus garlic oil compared to those who did not receive garlic
oil. This situation was seen after 90 days of treatment, especially in the recrudescence phase.\textsuperscript{23} Research conducted in 2004 showed allicin at a concentration of 30 g/ml in vitro was found to be able to effectively inhibit parasite development without damaging normal erythrocytes.\textsuperscript{27}

**Figure 2. Chemical Structure of Allicin** \textsuperscript{22}

### 2.4 Quercetin

Quercetin, a type of flavonoid, belongs to a group of polyphenolic compounds that are widely contained in plants. Quercetin, like a flavonoid in general, has been widely proven to have anti plasmodium effects. One study showed that quercetin has an inhibitory concentration of 50\% (IC\textsubscript{50}) with a high inhibitory potential equivalent to chloroquine control.\textsuperscript{8,28} Quercetin is one of major compounds found in garlic, and has been associated with the inhibitory effect of the intraerythrocyte phase of plasmodium.\textsuperscript{8,29} Other studies have identified quercetin as an active ingredient that exerts anti plasmodium effect by inhibiting the heme polymerization mechanism using mass spectroscopic analysis techniques.\textsuperscript{28} In the intraerythrocyte phase. *Plasmodium* uses hemoglobin as a nutrient. Metabolism products in the form of free heme are toxic to parasites, so there needs to be polymerization of free heme.\textsuperscript{30,31} The role of the active ingredient Quercetin on the development of malaria is shown through the mechanism of formation of complexes with heme so that heme cannot undergo polymerization and remains toxic to parasites.\textsuperscript{28}

**Figure 3. Chemical Structure of Quercetin** \textsuperscript{8}

### 3. Conclusion

Garlic (*Allium sativum*) is a plant that is easily found in various places and has active ingredients that can be used as medicinal ingredients. The active substances allicin, ajoene, and quercetin in garlic have the potential to be used to inhibit the development of plasmodium effectively and safely for the host.

**Suggestion**

Further research needs to be done through clinical trials so that the active substance in garlic can be used as an effective and safe malaria treatment.

**Conflict of Interest**

The author declares that there is no conflict of interest in this study.

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