International Journal of Engineering Business and Social Science
Vol. 3 No. 02, November-December 2024, pages: 223-232
e-ISSN: 2980-4108, p-ISSN: 2980-4272
https://ijebss.ph/index.php/ijebss

Effectiveness of Andrographis Paniculata Leaf Extract as an Antimalarial Through Plasmodium Heme Polymerization Inhibition

Brian Limantoro[1], Jovan Dewanta Nathanael[2], Devita Aulia Athifa[3], Felice Kanaya Chandra[4]*, Assyifa Aulia Zahra[5], Muhammad Imam Rizqi Ramadhan[6], Genova Alfonso[7], Irhentya Dwi Putri Lestari[8], Isma Dian Artika[9], Nadia Zelda Maulida Arisanti[10], Reinhan Djunaedi[11], Kenley Nathanael[12]
Airlangga University, Indonesia
Airlangga University, Indonesia
Udayana University, Indonesia
Email: felice.kanaya.chan-2023@fkg.unair.ac.id

Corresponding : Felice Kanaya Chandra

Keywords
andrographolide, genetic resistance, heme polymerization, malaria, Plasmodium

Abstract
Malaria is a significant public health challenge in the tropics, including Indonesia. The disease is caused by the Plasmodium parasite which is transmitted through the bite of the Anopheles mosquito. Although various antimalarial drugs have been developed, drug resistance by Plasmodium has led to a decrease in the effectiveness of conventional treatments such as chloroquine and artemisinin. Therefore, the exploration of alternative treatment is important, including the use of medicinal plants such as Andrographis paniculata (sambiloto) which contains the active compound andrographolida.
This study aims to analyze the potential of Andrographis paniculata as an antimalarial agent through inhibition of heme polymerization in Plasmodium parasites. The research approach used is a literature review by collecting literature from reputable journals published in the last decade. The data were analyzed descriptively to identify the mechanism of action, effectiveness, and pharmacological implications of sambiloto in the treatment of malaria.
The results showed that sabiloto leaf extract, especially andrographolide compounds, had the ability to inhibit heme polymerization, which is important in the life cycle of Plasmodium. Laboratory tests showed that sambiloto extract had an IC50 that was close to the effectiveness of standard drugs such as chloroquine, with low toxicity. In addition, proper extraction methods can improve the stability and effectiveness of these active compounds. The implication of this study is the importance of developing a standard formulation of Andrographis paniculata extract as an alternative or complementary to antimalarial therapy.

1 Introduction

Malaria remains a formidable public health challenge in several developing countries located in the tropics, including Southeast Asian countries such as Indonesia. In 2019, malaria prevalence was recorded at 227 million confirmed cases across Africa, South Asia, and Southeast Asia, with this number increasing to 241 million cases in 2020 (World Health Organization Press, 2021). In Indonesia, malaria continues to be rampant,

especially on the islands of Sumatra and Kalimantan (World Health Organization Press, 2021). This tropical disease is caused by about 250 species of protozoan parasites of the genus Plasmodium, which are mainly transmitted by vector insects, especially mosquitoes. Of these 250 protozoan species, 27 have been identified to be capable of infecting various primates around the world (A. Martinelli, 2018).

In humans, protozoan species responsible for malaria include Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae (Lempang, 2022). Among them, Plasmodium vivax emerged as the leading cause of malaria in Southeast Asia, making it the second most common malaria species globally (World Health Organization Press, 2016). The parasite initially attacks the liver, where it develops into sporozoites transmitted by the Anopheles mosquito. Once inside the human host, these sporozocytes, initially single-nucleated, mature into schizons with multiple cores, eventually producing thousands of merozoites. These merozoites enter the bloodstream, infect erythrocytes and disrupt the red blood cell cycle (Ernest et al., 2018). In addition, sporozoites remaining in the liver can interfere with innate immunity, leading to an abnormal response to external stress and increased susceptibility to infection, which can manifest as early symptoms of malaria. At this stage, the initial symptoms of malaria can be mitigated from progression to a more severe form through the administration of primaquine, a drug with the ability to eliminate hypnozoites, an inactive form of sporozoites within hepatocytes in the liver (Ernest, 2018).

Several therapeutic methods have been developed for the treatment of malaria; However, the effectiveness of these treatments can diminish over time due to the appearance of resistance. This resistance arises through a process of selective pressure applied to individual members of a particular species by certain drugs (Tantiamornkul, 2018) Chloroxine has been widely used as a therapeutic intervention for malaria globally and in Indonesia. Also known as 4-aminoquinoline, chloroquine is highly effective in treating malaria and is cost-effective. Various mechanisms of action of chloroquine have been discussed, with the most widely accepted mechanism being the inhibition of beta-hematin formation within parasitic digestive vacuoles (Olafson, 2015). Hematin crystallization is an important detoxification mechanism for malaria parasites, targeting the breakdown of hemoglobin-derived heme. This process is the main target for quinoline-based antimalarial drugs (Olafson, 2015). Malaria parasites that infect red blood cells catabolize hemoglobin, releasing heme Fe(II), which is then oxidized to hematin Fe(III) and absorbed into crystalline hemozoin. Resistance to chloroquine has made it less effective, requiring modifications to improve the efficacy of quinoline-based drugs against increasingly complex malaria strains. Today, quinolin derivatives, such as ferroquine (still in clinical trials) and amodiaquine (clinically approved), have been developed. Scientific evidence suggests that significant and precise modifications to chloroquine, especially at its site of introduction, can address the problem of chloroquine resistance (TN Wells, 2015; Dola et al., 2017).

In Indonesia, abundant natural resources, including medicinal plants, offer promising alternatives to malaria treatment. One such plant is Cinchona succirubra Pav. Ex Klotzsch, known for its quinine rich bark, an alkaloid with a content of more than 7% (Ministry of Health of the Republic of Indonesia, publication date not available). Kinin is effective against all species of Plasmodium and functions as schizontosides and gametocides, making it a frequent recommendation for the treatment of malaria. The process of utilizing quinine from cinchona bark involves isolating the desired alkaloids, extracting quinine using appropriate chemical solvents, and identifying and characterizing quinine alkaloids (Giri, 2020)

However, malaria remains a significant public health threat in Indonesia, especially in remote areas such as Papua, East Nusa Tenggara, and Kalimantan, which report the highest incidence of malaria each year. Based on a report by the Indonesian Ministry of Health, despite ongoing eradication efforts, Indonesia recorded more than 200 thousand cases of malaria in 2023, with some regions showing an increase in prevalence (Ministry of Education and Culture, 2023)

In addition, resistance to treatment is a growing challenge. Genetic mutations in Plasmodium falciparum have affected the effectiveness of several therapies, including kinin. This phenomenon emphasizes the need for new treatment alternatives that are more adaptive to drug-resistant malaria strains. On the other hand, overharvesting and global dependence on cinchona have led to a significant decline in functional cinchona forests. This dependence is particularly acute in tropical and subtropical regions, where cinchona is considered the only effective antimalarial crop (B Wasis, 2020).

Therefore, exploring and developing alternative medicinal plants for malaria treatment is essential to reduce dependence on cinchona, overcome drug resistance, and ensure sustainable and efficient management of malaria in the future. Recently, Andrographis paniculata, commonly known as sambiloto, has attracted significant attention as a potential alternative to malaria therapy. This medicinal plant that belongs to the Acanthaceae family and was originally found in China is known in traditional medicine as Chuan Xin Lian (EO

Jawa La, 2019). The therapeutic properties of sambiloto are attributed to its various constituents, which improve immune function by acting as an immunomodulator. These include tannins, saponins, flavonoids, and lactones, especially andrographolides (EO Jawa La, 2019).

Andrographolide is the main active compound in sambioto leaves, known for its hepatoprotective effects, which protect liver hepatocytes. It also exhibits anti-inflammatory, antipyretic, and antimalarial properties by inhibiting the growth of Plasmodium parasites such as Plasmodium berghei and Plasmodium falciparum. In addition, andrographolide has an antimicrobial effect against various pathogens in the human body. In addition to andrographolide, other significant active compounds in sambiloto leaves include neoandrographolide, 14-deoxy 11,12-didehydroandrographolide, 14-deoxyandrographolide, isoandrographolide, homoandrographolide, andrographan, 19-β-D-glucoside, andrographosterol, and stigmasterol (Anas et al., 2020). The antimalarial effect on Plasmodium falciparum has been shown in vitro using ethanol extract of sambiloto herb (Resi, 2014). The development and innovation of sambiloto leaf extract into a standard herbal product faces challenges, especially during the conventional extraction process, which can lead to the degradation of andrographolide due to overheating or overheating and the esterification reaction with alcohol, reducing the pharmacological efficacy of the extract (Anas et al., 2020). Biochemically, andrographolide is a diterpenoid and flavonoid lactone that can be extracted from roots and leaves, with the chemical formula C20H30O5 and crystal appearance (NK Warditiani, 2014). Flavonoids are soluble in alcohol but not in water, requiring special consideration for extraction and isolation to ensure the production of high-quality standard herbal medicines. Research shows that andrographolide exhibits a variety of pharmacological effects, including inhibition of platelet activation factors, antiviral activity against Herpes simplex virus type 1, anti-cancer effects on TD-47 breast cancer cells, cholesterol reduction, and anti-inflammatory properties in rheumatoid arthritis (NK Warditiani, 2014).

Therefore, a revolutionary extraction method that maintains the chemical stability of andrographolide is essential to process sambiloto leaves into high-quality standard herbal products. One promising method is hydrotropic extraction, which uses hydrotropic agents and operates under low temperature and pressure conditions, minimizing the degradation of andrographolides, which are less soluble in water (Anas et al., 2020). Further pharmacological studies on the effects of sambiloto on malaria-causing parasites can be carried out after the herbal extract is obtained through this advanced extraction method.

2 Materials and Methods

Type of Research

This research is a type of qualitative descriptive research that aims to understand certain phenomena based on scientific literature and empirical data. The focus of the study is to analyze the potential and effectiveness of Andrographis paniculata leaf extract as an antimalarial agent.

Research Approach

The research approach used is literature review. The data analyzed came from relevant scientific articles, journals, and research reports, accessed through academic databases such as ScienceDirect, ResearchGate, and Google Scholar.

Population and Sample

The study population is all literature related to antimalarial and Andrographis paniculata published in the last ten years. Samples were taken using purposive sampling techniques, with selection criteria including: Research relevant to the treatment of malaria and the pharmacological properties of Andrographis paniculata. Articles published in reputable journals.

Data Collection Techniques

Data were collected through document studies, with the following steps: Searching for scientific articles using keywords such as "malaria," "Andrographis paniculata," and "inhibition of heme polymerization." Segment data based on topical relevance, methods, and research results. Validate sources by prioritizing literature from reputable journals.

Data Analysis Techniques

The data analysis technique is carried out qualitatively-descriptively through the following steps: Reading and understanding the selected literature. Identifying the mechanism of action of Andrographis paniculata in the treatment of malaria. Comparing the findings of the literature related to the antimalarial efficacy of Andrographis paniculata. Drawing conclusions based on patterns found in the literature.

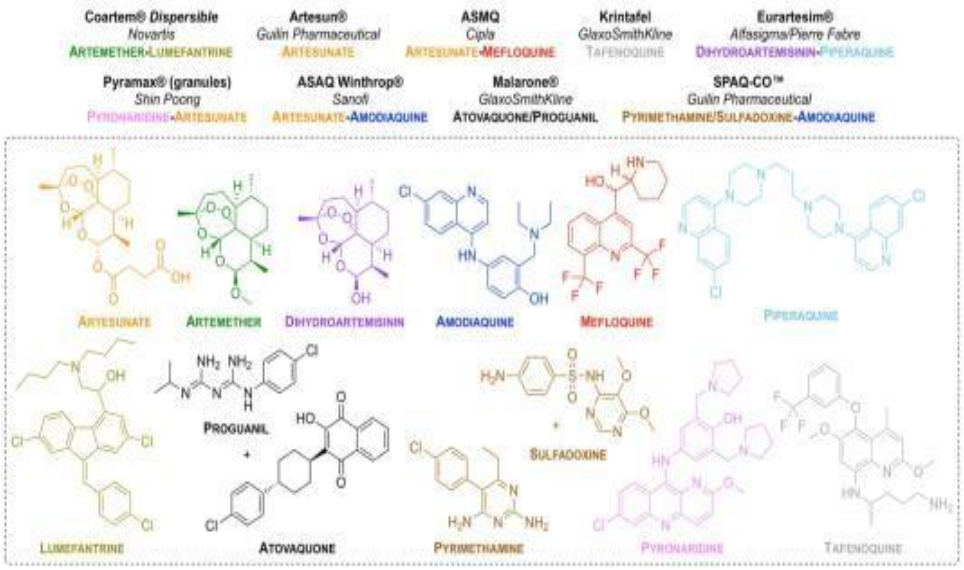
3 Results and Discussion

In contemporary times, the main antimalarial compounds known to control the growth of malaria parasites are chloroquine and artemisinin. These compounds inhibit the fundamental processes necessary for *Plasmodium* parasites to replicate and infect the host more aggressively during its maturation phase. Historically, chloroquine functions as an antimalarial by blocking the crystallization of beta-hematin within the host's red blood cells (NK Warditiani, 2014:Tse, 2019). Chloroquine, which comes from the quinine containing plant, *Cinchona*, was very effective in the past and remains famous today. However, resistance to chloroquine has developed in various *strains of Plasmodium*, including K1, 7GB, W2, Dd2 (Tse, 2019).

Regarding artemisinin, although the exact antimalarial mechanism is still somewhat unclear, it is believed that artemisinin produces free radicals after activation by the heme group of red blood cells, which causes oxidative damage to red blood cell proteins (Tse, 2019). A computational approach conducted a decade ago in 2013 aimed to elucidate the mechanism of artemisininin, with a focus on the identification of the heme group and the calcium ion transporter PfATP6, which is hypothesized to be a key mechanism (Shandilya, 2013). A subsequent study in 2015 revealed that artemisinin was associated with increased regulation of the unfolded protein response pathway (UPR), which correlates with reduced parasite development and acts as a potential inhibitor of the enzyme phosphatidilinositol-3-kinase in *Plasmodium falciparum* (S Mok, 2014 ; (Mbengue, 2015).

Despite the advancements, recent advances include semi-synthetic derivatives of artemisinin, such as artemether, artesunate, and arteeter. This derivative, as illustrated in Figure 1, is converted into the active metabolite dihydroartemisinin, which plays an important role in modern malaria treatment regimens (Tse, 2019). These innovations represent a significant step in the ongoing battle against malaria, reflecting advances in therapeutic strategies and the ongoing evolution of antimalarial medicine.

Figure 1. Formulations or Combinations of New Approved Antimalarial Drugs (Tse, Korsik, and Todd, (2019).



Due to increased resistance and reduced effectiveness, chloroquine and artemisinin can no longer function as stand-alone antimalarial treatments. To increase the efficacy of antimalarial drugs in targeting malaria parasites that thrive in red blood cells, alternative or complementary compounds are needed. Among these alternatives, andrographolide—a flavonoid compound found in *Andrographis paniculata* (sambiloto)—has shown significant pharmacological effects, including antimicrobial, antitumor, and antimalarial properties.

Andrographolide is extracted from sambiloto leaves or herbs through a special method, as conventional extraction techniques can lead to degradation of these compounds due to overheating (Anas et al., 2020). Its antioxidant properties contribute to its role as an antimicrobial and antitumor agent, with potential applications in treating early-stage breast cancer (Yunita, 2021). In unstable molecular conditions, where the electrons on the

outer shell are unpaired, substances such as reactive oxygen species (ROS) can form. ROS is highly reactive and can cause oxidative damage by targeting enzymes, lipid membranes, and DNA (Yunita, 2021). The accumulation of such radicals can interfere with cellular function, especially affecting unsaturated fatty acids in cell membranes, as depicted in Figure 2 (Nwazue, 2014). Antioxidant compounds, including andrographolide, can neutralize ROS by preventing the continuation of radical synthesis and facilitating the conversion of radicals into non-radical forms (Yunita, 2021). This mechanism is crucial for the application of sambiloto herb extract in the fight against malaria, specifically targeting the Plasmodium parasite in infected red blood cells.

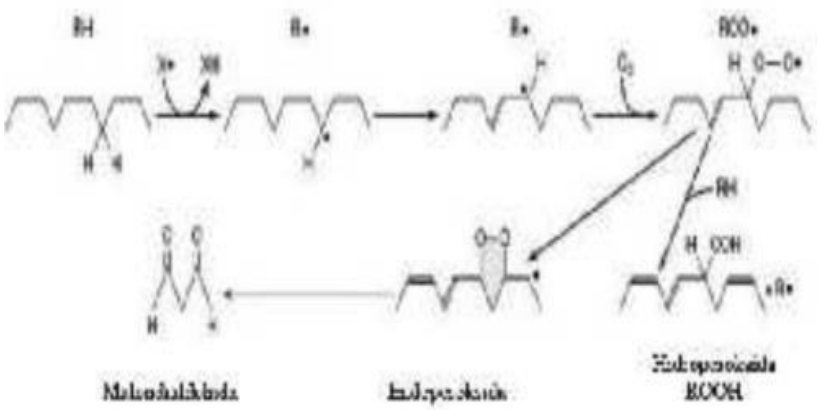


Figure 2. Oxidative Damage to Cell Membranes and Antioxidant Protection (Nwazue et al., 2014).

Historically, various plant extracts have been explored for their antimalarial potential. Important examples include *Phyllanthus amarus*, which has shown reliable antimalarial activity in antiplasmodium tests in vivo, and the ethanol fraction of garlic, which is reported to be more effective in inhibiting heme polymerization compared to n-hexane and ethyl acetate fractions N.R. Nwazue, (2014); S Manu, (2013). In this context, the application of sambioto herb extract, when processed with appropriate solvents based on its polarity, is expected to show performance in inhibiting heme polymerization and preventing the formation of hemozoins, which is beneficial for parasites (Septiana, Gianny, and Simanjuntak, 2017). To validate these findings, controls are crucial for comparison, using chloroquine, which has historically been shown to be effective against malaria (Septiana, Gianny, and Simanjuntak, 2017). Previous studies have shown that andrographolide, a key compound in sambiloto, inhibits heme polymerization with an IC50 value of 367 µM or about 128 µg/mL (Risdawati, 2014). Toxicity tests can be performed using the Saltwater Shrimp Lethality Test (BSLT), where the extract is considered toxic if the LC50 is less than 1000 µg/mL and non-toxic if the LC50 is greater than 1000 µg/mL (Mamatha, 2014). BSLT is a direct method of evaluating the cytotoxicity of a substance by assessing its ability to kill saltwater shrimp (*Artemia salina nauplius*), as depicted in Figure 3. Heme polymerization inhibition is calculated by subtracting the test substance's hematin levels from the control and dividing this difference by the control hematin levels, then multiplying by 100%.

Table 1. Presenting observations of the effectiveness of various candidates of sambiloto leaf extract modified with specific compounds at different concentrations, showing variations in cytotoxicity levels in IC50 parameters.

Bahan uji	Konsentrasi (µg/mL)	Kadar hematin (µM) ±SD	Penghambatan (%)	IC_{50} (µg/mL) ±SD
Ekstrak n-heksan	125	133,63±0,56	0,86	2196,57±94,16
	250	105,29±5,94	21,87	
	500	100,83±5,31	25,16	
	1000	94,63±1,38	29,78	
	2000	74,88±2,13	44,43	
Ekstrak etil asetat	125	112,63±2,13	16,42	1235,54±8,79
	250	109,29±9,94	18,91	
	500	88,58±6,44	34,27	
	1000	54,29±2,31	59,71	
	2000	51,25±1,63	61,96	
Ekstrak etanol 70%	125	126,21±7,69	6,33	1157,24±18,61
	250	108,00±5,38	19,86	
	500	77,13±0,50	42,76	
	1000	53,00±0,88	60,67	
	2000	46,88±1,75	65,21	

Table 2. Displaying the results of the cytotoxicity test of sambiloto leaf extract candidate at LC50 parameters.

Klorokuin	62,5	117,88±1,19	12,52	698,85±6,93
	125	94,29±0,19	30,02	
	250	82,54±0,31	38,74	
	500	70,46±1,06	47,71	
	1000	56,71±0,26	57,92	
Akuades		134,75±0,38	0,00	

Jenis ekstrak	LC_{50} (µg/mL)
n-heksan	1.155,79
Etil asetat	1.133,89
Etanol 70%	5.229,15

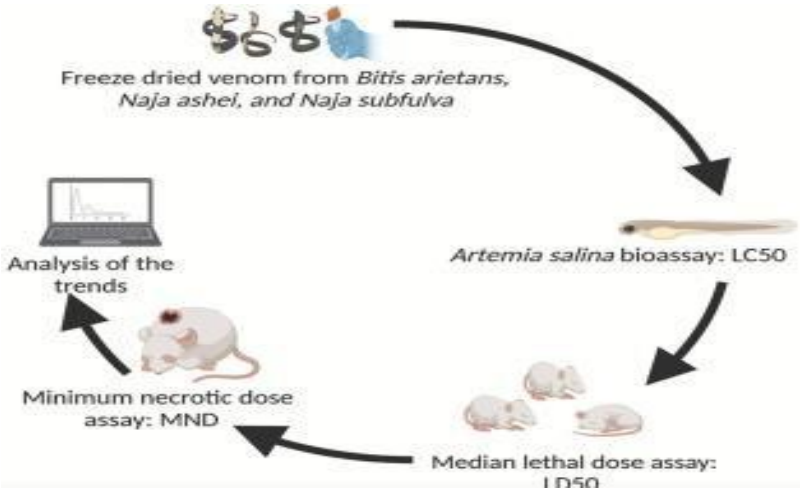


Figure 3. Saltwater Shrimp Lethality Test Methodology (BSLT) for Cytotoxicity Evaluation.

Based on the results presented in Tables 1 and 2, it is evident that the three types of sambiloto leaf extract show significant potential in inhibiting heme polymerization. Among them, 70% ethanol extract shows the highest effectiveness. This is due to the higher concentration of chemical compounds compared to n-hexane and ethyl acetate extracts. The same study revealed that flavonoids, saponins, and tannins—present exclusively in 70% ethanol extracts—play an important role in antimalarial activity. For example, flavonoids can inhibit heme polymerization by forming quercetin-heme complexes, tannins can inhibit the growth of Anopheles mosquitoes, and saponins also contribute to antimalarial effects (Okumu, 2021).

Further research supports these findings, highlighting the antimalarial ability of andrographolide extracted from sabiloto leaves. A study by (Widyawaruyanti et al., 2014) used andrographolide extracted from dried sambiloto leaves and formulated into tablets. In this study, in vivo tests were carried out on mice infected with the strain of *Plasmodium berghei* ANKA, the parasite that causes malaria. Andrographolide tablets were developed by combining two fractions of ethanol extracts: Fraction A, obtained by fractionation with ethyl acetate, and Fraction B, purified from Fraction A. Although both fractions contain diterpenoid lactones, their physical and chemical properties are very different. Fraction A, which is characterized by a dark green sticky substance, appears to reduce the efficacy of the active substance and reduce bioavailability in vivo (Widyawaruyanti et al., 2014).

The tablets are prepared using two unique techniques: wet granulation and dry dispersion, resulting in three different phytopharmaceutical products. These include Tablet I (wet granulation of Fraction A), Tablet II (wet granulation of Fraction B), and Tablet III (dry dispersion of Fraction B) (Widyawaruyanti et al., 2014). The antimalarial activity of these tablets was evaluated using the Peter test, a 4-day suppression test, in which the tablets were administered orally to infected rats at a dose of 12.55 mg andrographolide/kg suspended in a 0.5% CMCNa solution. The mice that received only 0.5% of CMCNa served as a negative control.

On the fifth day, blood samples were taken from each mouse to determine the level of parasitemia by counting the number of infected red blood cells among a thousand randomly selected cells under a microscope. Parasitemia inhibition is calculated by reducing the proportion of infected red blood cells in the test sample from a negative control, with the result expressed as a percentage of inhibition.

A study by Widyawaruyanti et al. (2014) showed that all three tablet formulations inhibited the growth of parasites in the blood, albeit with varying degrees of effectiveness, as evidenced by different percentage inhibition levels. The andrographolide dose was consistent across all treatments at 25.10 mg/kg/day (Widyawaruyanti et al., 2014).

Table 3. provides detailed observations on the antiplasmodial activity of Tablets I, II, and III, reflecting their various efficacy in inhibiting malaria parasites.

Sample	Active substance	Andrographolide dose (mg/kg bw/day)	Average parasitemia (%)	Average inhibition (%)
Tablet I	AP fraction A	25.10	3.37±0.29	70.15
Tablet II	AP fraction B	25.10	2.85±0.48	78.16
Tablet III	AP fraction B	25.10	2.96±0.41	80.35
Negative control	-	-	10.85±1.54	-

Additional Information:

a. The data were expressed as mean ± standard deviation for five mice per group with F = 53.789.

b. $P < 0.001$; comparison with control.

Interpretation:

- **Tablets I, II, and III** showed significant inhibition of parasitemia compared to the control group, as indicated by a P-value of less than 0.001.
- **Tablet I**, **Tablet II**, and **Tablet III** showed varying levels of efficacy, as evidenced by different percentages of average parasitemia. Statistical analysis confirms that the difference is significant.

This detailed table and additional information underscores the effectiveness of andrographolide-containing tablets in inhibiting the growth of malaria parasites, demonstrating their potential as a therapeutic option in the treatment of malaria. Based on the data presented in Table 3 and analyzed using a one-way ANOVA at a confidence level of 95%, significant differences were observed between Tablet I and Tablet II, as well as between Tablet I and Tablet III. In contrast, the difference between Tablet II and Tablet III is not statistically significant. These findings suggest that the main factor influencing the antimalarial activity of tablets is the concentration of the active substances they contain, not the method of preparation of the tablets. In particular, Tablet I, which is based on less pure extracts, shows a more pronounced antimalarial effect compared to Tablets II and III. Tablets II and III use andrographolide extracted from a purer source (AP Fraction B Extract), which increases its concentration and potency. This shows that the purification process significantly increases the effectiveness of andrographolide as an antimalarial agent. As a result, higher concentrations of active andrographolides in Tablets II and III resulted in greater antimalarial efficacy, highlighting the importance of purification of the extract in maximizing the therapeutic potential of the tablets.

4 Conclusion

The sambiloto plant, although not yet widely recognized, has shown significant potential in vitro and in vivo studies as an alternative treatment for malaria, comparable to the chloroquine of the cinchona plant. Sambiloto leaves or herbs are able to inhibit heme polymerization in Plasmodium parasites, especially Plasmodium falciparum, the main cause of tropical malaria, with high efficacy because it has not been affected by genetic resistance factors. Andrographolide compounds, found in sambioto leaves or herbs, offer a variety of therapeutic benefits. In addition to acting as an antioxidant that protects the body from oxidative damage, andrographolide also functions as an antimicrobial in several vital organs, as an antitumor agent in early-stage breast cancer, and as a hepatoprotector that protects the liver.

Therefore, the extraction of andrographolide should be done using proper techniques that follow its physical and chemical characteristics to ensure that the resulting extract is effective in enhancing the antimalarial mechanism. The most effective extracts for treating malaria are those with high concentrations of

andrographolide. This can be achieved through purification methods that remove unwanted compounds, resulting in an optimal andrographolide composition. Thus, the extract can provide maximum pharmacological effects with minimal risk of toxicity and unwanted side effects. Further research in clinical trials is needed to ascertain the procedure and safety of sambiloto leaf extract when consumed by humans, as well as its effectiveness in the human body. Testing on individuals of simple complexity provides only a basic frame of reference, but additional studies are needed to validate its comprehensive efficacy and safety in more complex and diverse human populations.

5. References

A Mamatha, lethal test of saltwater shrimp Andrographis paniculata, Journal of Pharmaceutical and Technological Research (7), (2014), 743–5

A Mbengue, S Bhattacharjee, T Pandharkar, Liu H, G Estiu, R.V. Stahelin, Molecular mechanism of artemisinin resistance in malaria Plasmodium falciparum, Nature (2015), 520:683–7

A Shandilya, S Chacko, B Jayaram, I Ghosh, A plausible mechanism for artemisinin antimalarial activity: a computational approach, International Journal of Scientific Reports (3), (2013)

A Widyawaruyanti, M Asrory, W Ekasari, D Setiawan, A Radjaram, L Tumewu, AF Hafid, Antimalarial activity in vivo tablets Andrographis paniculata, Procedia Chemistry (13), (2014), 101–104, DOI: 10.1016/j.proche.2014.12.012

A. Martinelli, R. Culleton, Non-human primate malaria parasites: out of the forest and into the laboratory, Parasitology 145 (2018),41–54, https://doi.org/10.1017/S0031182016 001335.

B Wasis, E Sandra, Ecological study of quinine tree (Cinchona spp.) and its benefits in overcoming the spread of malaria 1 – Ecological study of quinine tree (Cinchona spp.) and its benefits in overcoming the spread of malaria, Bogor Agricultural University (2020),10.13140/RG.2.2.31150.873 62

E Septiana, D Gianny, P Simanjuntak, Toxicity and antimalarial activity through in vitro inhibition of heme polymerization of sambiloto leaf extract (Andrographis paniculata), Health Research and Development Media (2017), 255–262, http://dx.doi.org/10.22435/mpk.v27i4. 6499.255-262

E Yunita, Mechanism of action of andrographolides from sambiloto as antioxidant compounds, Herb-Medicine Journal (2021), 43–56, 10.30595/hmj.v4i1.8825

E.G. Tse, M Korsik, M. H. Todd, Past, present, and future of anti-malarial drugs, Journal of Malaria (2019), https://doi.org/10.1186/s12936-019-2 724-z

EM Resi, Effect of antimalarial herbal sambiloto (Andrographis paniculata Nees) on developmental morphological changes and parasites of Plasmodium falciparum, Journal of Health Info 12(1), (2014), 661–671

EO Jawa La, PDM Kurnianta, Study of active compounds and safety of traditional medicinal plants in Indonesia as an alternative to malaria treatment, Acta Holistica Pharmaciana 1(1), (2019), 33–43

J.M. Muema, J.L. Bargul, S.G. Nyanjom, J.M. Mutunga, S.N. Njeru, Potential of Camelia sinensis proanthocyanidins – rich fraction to control malaria mosquito populations through impaired larval development, Parasite Vectors (9), 2016

K Tantiamornkul, T Pumpaibool, Prevalence of molecular markers of drug resistance in Plasmodium vivax from the border region of Thailand in 2008 and 2014, International Journal of Parasitology: Drugs and Drug Resistance 8 (2018) 229–237, https://doi.org/10.1016/j.ijpddr.2018.0 4.003

K.N. Olafson, M.A. Ketchum, J.D. Rimer, P.G. Vekilof, Mechanism of hematin crystallization and inhibition by chloroquine antimalarial drugs, Proc Natl Acad Sci 112 (2015), 4946–4951, https://doi.org/10.1073/pnas.1501023 112

M Ernest, C Hunja, Toll Like Receptor 2 PEG-Pam2Cys Agonist as Immunochemoprophylaxis and Immunochemotherapy against the liver stage and malaria parasite transmission, International Journal of Parasitology 8 (2018)451– 458, https://doi.org/10.1016/j.ijpd dr.2018.10.006

MEP Lempang, FK Dewayanti, Primate malaria: The emerging zoonotic malaria challenge in Indonesia, One Health 14 (2022) 100389,https://doi.org/10.1016/j.onehl t.2022.100389

Ministry of Health of the Republic of Indonesia. Materia Medika Indonesia, Volume IV. Jakarta: Ministry of Health of the Republic of Indonesia.

MO Okumu, JM Mbaria, JK Gikunju, PG Mbuthia, VO Madadi, FO Ochola, MS Jepkorir, Artemia salina as an animal model for the initial evaluation of snake venom-induced toxicity, Toxicon:X (12), (2021), https://doi.org/10.1016/j.toxcx.2021.1 00082

N.R. Nwazue, O Jacinta, B Wesley, Effects of malaria in vivo ethanol and coarse aqueous extract of Phyllantus amarus, World Essay Journal (1), (2014), 115–24

NK Warditiani, INK Widjaja, N.W. R. Noviyanti, Andrographolid isolation from Andrographis paniculata (Burm. f.) Ness using purification and crystallization methods, Udayana Pharmaceutical Journal (2014), 31–34

OO Ogunlana, Kim HS, Y Wataya, JO Olagunju, AA Akindahunsi, Tan NH, Antiplasmodial flavonoids from young twigs and leaves of Caesalpinia bonduc (Linn) Roxb, Journal of Chemical Pharmaceutical Research (7), (2015)

Prof. Giri, Identification and determination of quinine compound levels of quinine acetate fraction of quinine bark (Cinchona succirubra Pav Ex Klotzsch) by KLT – densitometry, Scientific Periodical of Indonesian Pharmacy Students Volume 7 Edition 2 (2020), 1–12, https://doi.org/10.48177/bimfi.v7i2.41

Risdawati, Mechanism of action of andrographolides from sambiloto (Andrographis paniculata, Nees) as antimalarial compounds: a study on the oxidative status of Plasmodium berghei ANKA, Jakarta: University of Indonesia (2014)

S Manu, R Deshmukh, KMN Prasad, V Trivedi, Screening and characterization of antimalarial heme polymerase inhibitors from garlic cloves, European Journal of Medical Plants (3), (2013), 474–84

S Mok, Ashley EA, Ferreira PE , Zhu L, Lin Z, Yeo T, Human malaria parasite population transcriptome reveals artemisinin resistance mechanism, Science (2014), 347:431–5

TN Wells, R Hooft van Huijsduijnen, Ferroquine: welcome to the next generation of antimalarials, Infectious Diseases Lancet 15 (2015), 1365–1366, https://doi.org/10.1016/S 1473-3099(15)00148-6

VR Dola, A Soni, P Awargal, K.S. Raju, Synthesis and evaluation of chiralally determined side chain variants of 7-chloro-4-aminoquinoline to overcome drug resistance in malaria chemotherapy, Antimicrobial Agents and Chemotherapy 61 (2017), https://doi.org/10.1128/aac.01152-16

World Malaria Report, Health Organization 2016, Geneva, 2016.

World Malaria Report, World Health Organization 2020, Geneva, 2020. https://www.who.int/publications/i/item

World Malaria Report, World Health Organization Press 2021, Geneva, 2021.https://who.int/publications/i/item/9789240040496.

Y Anas, RD Ratnani, L Kurniasari, I Hartati, Anti-Plasmodium Activity of Sambiloto Leaf Hydrotropy Extract (Andrographis paniculata Ness.) in vitro on Plasmodium falciparum strain G-2300 chloroquine-resistant, Journal of Pharmaceutical Sciences and Clinical Pharmacy 17(1), (2020), 01–07