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Effectiveness of Sambiloto leaf extract as an antimalarial through plasmodium heme polymerization inhibition: A review

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Abstract

Background: Malaria remains a significant and persistent public health challenge in Indonesia and other tropical regions. This disease is caused by the Plasmodium parasites, which are transmitted to humans through the bite of Anopheles mosquitoes and subsequently infect hepatocytes in the liver. Despite the long history of antimalarial drug development, resistance by Plasmodium species has led to diminishing effectiveness of conventional treatments such as chloroquine and artemisinin. Consequently, there is renewed interest in herbal remedies, such as *Andrographis paniculata* (sambiloto), which may offer alternative therapeutic options.

Objective: To elucidate the pharmacological potential of sambiloto leaf extract as an antimalarial agent.

Method: This study involves a comprehensive literature review of scientific reports, including practical reports, case studies, systematic reviews, and meta-analyses, published within the past decade. The review was conducted through reputable scientific databases, including ScienceDirect, ResearchGate, and Google Scholar.

Result: The pharmacological efficacy of Andrographis paniculata leaf extracts is attributed to their high concentration of andrographolides, which have demonstrated the capability to inhibit heme polymerization in Plasmodium parasites. Both in vitro and in vivo studies have confirmed the safety and non-toxic nature of these extracts. Given the current state of genetic resistance in Plasmodium, the use of andrographolide extracts presents a promising avenue for effective malaria treatment.

Conclusion: The concentrated andrographolide extract from Andrographis paniculata leaves exhibits significant antimalarial activity by targeting and inhibiting heme polymerization in Plasmodium. This suggests that it could serve as a viable alternative or complement to existing antimalarial therapies

Keywords: Andrographolide; Genetic resistance; Heme polymerisation; Malaria; Plasmodium

1. Introduction

Malaria remains a formidable public health challenge in several developing countries situated in tropical regions, including Southeast Asian nations such as Indonesia. In 2019, the prevalence of malaria was recorded at 227 million confirmed cases across Africa, South Asia, and Southeast Asia, with this number rising to 241 million cases in 2020 (1). In Indonesia, malaria continues to be prevalent, particularly on the islands of Sumatra and Kalimantan (2). This tropical disease is caused by approximately 250 species of protozoan parasites from the *Plasmodium* genus, which are primarily

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transmitted by vector insects, notably mosquitoes. Out of these 250 protozoan species, 27 have been identified as capable of infecting various primates worldwide (3).

In humans, the protozoan species responsible for malaria include *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* (4). Among these, *Plasmodium vivax* emerges as the predominant cause of malaria in Southeast Asia, making it the second most prevalent malaria species globally (5). The parasite initially invades the liver, where it develops into sporozoites transmitted by Anopheles mosquitoes. Once inside the human host, these sporozoites, initially single-nucleated, mature into schizonts with multiple nuclei, eventually producing thousands of merozoites. These merozoites enter the bloodstream, infecting erythrocytes and disrupting the red blood cell cycle (6). Additionally, sporozoites remaining in the liver can impair innate immunity, leading to abnormal responses to external stressors and increased susceptibility to infections, which can manifest as early symptoms of malaria. At this stage, early symptoms of malaria can be mitigated from progressing to more severe forms through the administration of primaquine, a drug with the capability to eliminate hypnozoites, the dormant form of sporozoites within hepatocytes in the liver (6).

Several therapeutic methods have been developed for the treatment of malaria; however, the effectiveness of these treatments can diminish over time due to the emergence of resistance. This resistance arises through a process of selective pressure applied to individual members of a particular species by specific drugs (7). Chloroquine 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 chloroquine's action have been discussed, with the most widely accepted mechanism being the inhibition of beta-hematin formation within the parasite's digestive vacuole (8). Hematin crystallization is a crucial detoxification mechanism for malaria parasites, targeting the breakdown of heme derived from hemoglobin. This process is a primary target for quinoline-based antimalarial drugs (8). Malaria parasites that infect red blood cells catabolize hemoglobin, releasing Fe(II) heme, which is then oxidized to Fe(III) hematin and sequestrated into crystalline hemozoin. Resistance to chloroquine has rendered it less effective, necessitating modifications to improve the efficacy of quinoline-based drugs against increasingly complex malaria strains. Today, derivatives of quinoline, such as ferroquine (still in clinical trials) and amodiaquine (clinically approved), have been developed. Scientific evidence suggests that significant and precise modifications to chloroquine, particularly at its recognition site, can address chloroquine resistance issues (9,10).

In Indonesia, abundant natural resources, including medicinal plants, offer promising alternatives for malaria treatment. One such plant is *Cinchona succirubra* Pav. Ex Klotzsch, known for its bark rich in quinine, an alkaloid with more than 7% content (11). Quinine is effective against all Plasmodium species and serves as both a schizontocide and gametocide, making it a frequent recommendation for malaria treatment. The process of utilizing quinine from cinchona bark involves isolating the desired alkaloids, extracting quinine using suitable chemical solvents, and identifying and characterizing the quinine alkaloids (12). However, due to excessive harvesting and global reliance on cinchona, there has been a significant decline in functional cinchona forests. This reliance is particularly acute in tropical and subtropical regions, where cinchona is perceived as the sole effective antimalarial plant (13). Therefore, exploring and developing alternative medicinal plants for malaria treatment is crucial to reduce dependency on cinchona and ensure continued effective and efficient malaria management in the future. Additionally, genetic strain resistance can impact the efficacy of treatments within the human body.

Recently, *Andrographis paniculata*, commonly known as sambiloto, has garnered significant attention as a potential alternative for malaria therapy. This medicinal plant, which belongs to the Acanthaceae family and was originally discovered in China, is known in traditional medicine as Chuan Xin Lian (14). The therapeutic properties of sambiloto are attributed to its various constituents, which enhance immune function by acting as immunomodulators. These include tannins, saponins, flavonoids, and lactones, particularly andrografolides (14).

Andrographolide is the primary active compound in the leaves of sambiloto, known for its hepatoprotective effects, which safeguard 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*. Additionally, andrografolide has antimicrobial effects against various pathogens within the human body. Besides andrografolide, 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 (15). The antimalarial effects against *Plasmodium falciparum* have been demonstrated in vitro using ethanol extracts of sambiloto herb (16). The development and innovation of sambiloto leaf extracts into standardized herbal products face challenges, particularly during the conventional extraction process, which can lead to the degradation of andrografolide due to overheating or excessive heating and esterification reactions with alcohol, reducing the pharmacological efficacy of the extract (15). Biochemically, andrografolide is a diterpenoid

lactone and flavonoid that can be extracted from both roots and leaves, with a chemical formula of $C_{20}H_{30}O_5$ and a crystalline appearance (17). Flavonoids are soluble in alcohol but not in water, requiring special considerations for extraction and isolation to ensure the production of a high-quality standardized herbal medicine. Research indicates that andrografolide demonstrates various pharmacological effects, including inhibition of platelet-activating factor, 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 (17).

Therefore, revolutionary extraction methods that preserve the chemical stability of andrografolide are essential for processing sambiloto leaves into high-quality standardized herbal products. One promising method is hydrotropic extraction, which uses hydrotropic agents and operates under low temperature and pressure conditions, minimizing the degradation of andrografolide, which is less soluble in water (15). Further pharmacological studies on sambiloto's effects on malaria-causing parasites can be conducted once the herb's extracts are obtained through these advanced extraction methods.

2. Material and methods

The discussion on the potential of *Andrographis paniculata* (sambiloto) as an antimalarial agent was conducted through a comprehensive literature review of reputable scientific articles. The sources were accessed through established academic databases, including ScienceDirect, ResearchGate, and Google Scholar. This review focused on general information about malaria, common treatments provided to malaria patients, the effectiveness of antimalarial drug components in combating malaria parasites, and the potential of sambiloto in the context of its biochemical and pharmacological aspects as an alternative antimalarial solution. The selected articles for this review were published within the past decade, specifically from 2013 onwards, to ensure the relevance and currency of the information. Keywords and phrases that facilitated the search included: malaria and its prevalence, particularly in Southeast Asia and Indonesia; quinine as an antimalarial; pharmacological effects of quinoline derivatives; *Andrographis paniculata* and its medicinal properties; and the mechanisms of action of sambiloto herb extracts in malaria treatment. A total of thirty-one articles were utilized in this literature review, drawn from a range of sources to provide a well-rounded perspective on the subject.

3. Results and discussion

In contemporary times, the primary antimalarial compounds known for controlling the growth of malaria parasites are chloroquine and artemisinin. These compounds inhibit fundamental processes necessary for the *Plasmodium* parasite to replicate and infect hosts more aggressively during its maturation phase. Historically, chloroquine functioned as an antimalarial by obstructing the crystallization of beta-hematin within the red blood cells of the host (17,18). Chloroquine, derived from the quinine-containing plant, *Cinchona*, was highly effective in the past and remains renowned today. However, resistance to chloroquine has developed in various *Plasmodium* strains, including K1, 7GB, W2, Dd2, among others (18).

Regarding artemisinin, while its precise antimalarial mechanism remains somewhat unclear, it is believed that artemisinin generates free radicals upon activation by heme groups from red blood cells, leading to oxidative damage to the red blood cell proteins (18). A computational approach undertaken a decade ago in 2013 aimed to elucidate the mechanism of artemisinin, focusing on the identification of heme groups and the calcium ion transporter PfATP6, which was hypothesized to be a key mechanism (19). Subsequent studies in 2015 revealed that artemisinin is associated with the upregulation of the unfolded protein response (UPR) pathway, which correlates with reduced parasite development and acts as a potential inhibitor of the phosphatidylinositol-3-kinase enzyme in *Plasmodium falciparum* (20,21).

Despite the progress, the most recent advancements include semi-synthetic derivatives of artemisinin, such as artemether, artesunate, and arteether. These derivatives, as illustrated in Figure 1, are transformed into the active metabolite dihydroartemisinin, which plays a crucial role in modern malaria treatment regimens (18). These innovations represent significant strides in the ongoing battle against malaria, reflecting advancements in therapeutic strategies and the continuous evolution of antimalarial treatments.

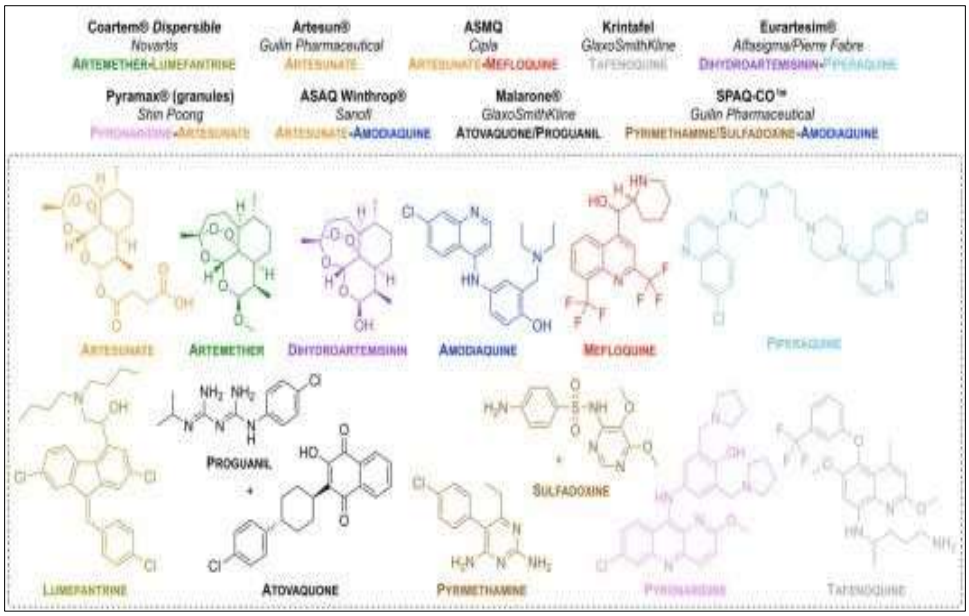

Figure 1 Approved Formulations or Combinations of New Antimalarial

Due to increasing resistance and diminished effectiveness, chloroquine and artemisinin can no longer serve as standalone antimalarial treatments. To enhance the efficacy of antimalarial drugs in targeting malaria parasites that thrive within red blood cells, alternative or complementary compounds are required. Among such alternatives, andrographolide—a flavonoid compound found in *Andrographis paniculata* (sambiloto)—has demonstrated notable pharmacological effects, including antimicrobial, antitumor, and antimalarial properties.

Andrographolide is extracted from the leaves or herb of sambiloto through specialized methods, as conventional extraction techniques may lead to degradation of this compound due to overheating (15). Its antioxidant properties contribute to its role as an antimicrobial and antitumor agent, with potential application in treating early-stage breast cancer (22). In unstable molecular conditions, where electrons on the outer shell are unpaired, substances like reactive oxygen species (ROS) can form. ROS are highly reactive and can lead to oxidative damage by targeting enzymes, lipid membranes, and DNA (22). The accumulation of such radicals can disrupt cellular functions, particularly affecting unsaturated fatty acids in cell membranes, as depicted in Figure 2 (23). Antioxidant compounds, including andrographolide, can neutralize ROS by preventing the continuation of radical synthesis and facilitating the conversion of radicals to non-radical forms (22). This mechanism is crucial for the application of sambiloto herb extracts in combating malaria, specifically targeting Plasmodium parasites within infected red blood cells.

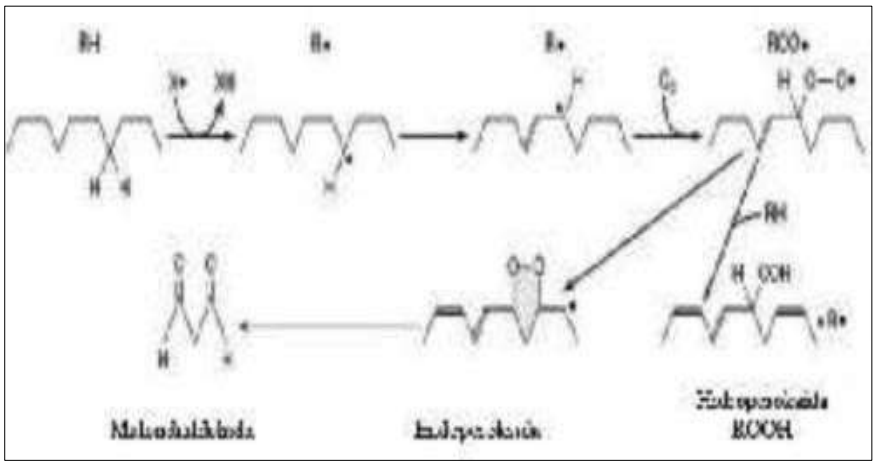

Figure 2 Oxidative Damage to Cell Membranes and Antioxidant Protection

Historically, various plant extracts have been explored for their antimalarial potential. Notable examples include *Phyllanthus amarus*, which has demonstrated reliable antimalarial activity in in vivo antiplasmodium tests, and

ethanolic fractions of garlic, which are reported to be more effective in inhibiting heme polymerization compared to n-hexane and ethyl acetate fractions (23,24). In this context, the application of sambiloto herb extracts, when processed with appropriate solvents based on their polarity, is expected to exhibit performance in inhibiting heme polymerization and preventing the formation of hemozoin, which benefits the parasite (25). To validate these findings, a control is essential for comparison, utilizing chloroquine, which has historically been proven effective against malaria (25). Previous research has shown that andrographolide, a key compound in sambiloto, inhibits heme polymerization with an IC50 value of 367 µM or approximately 128 µg/mL (26). Toxicity testing can be conducted using the Brine Shrimp Lethality Test (BSLT), where an 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 (27). The BSLT is a straightforward method to evaluate the cytotoxicity of a substance by assessing its ability to kill brine shrimp (*Artemia salina nauplius*), as depicted in Figure 3. The inhibition of heme polymerization is calculated by subtracting the hematin levels of the test substance from the control and dividing this difference by the control hematin levels, then multiplying by 100%.

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

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 Displays the results of cytotoxicity tests for sambiloto leaf extract candidates in LC50 parameters

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

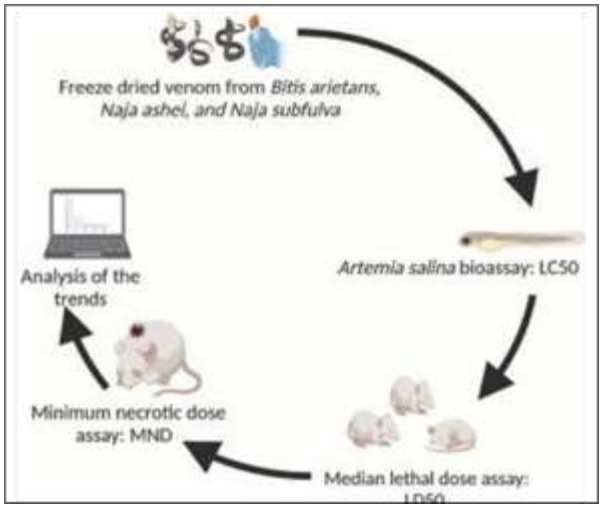

Figure 3 Brine Shrimp Lethality Test (BSLT) Methodology for Cytotoxicity Evaluation (28)

Based on the results presented in Tables 1 and 2, it is evident that all three types of sambiloto leaf extracts exhibit significant potential in inhibiting heme polymerization. Among these, the 70% ethanol extract demonstrates the highest effectiveness. This is attributed to its higher concentration of chemical compounds compared to the n-hexane and ethyl acetate extracts (25). The same study revealed that flavonoids, saponins, and tannins—present exclusively in the 70% ethanol extract—play crucial roles in antimalarial activity. For instance, 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 (29,30).

Further research supports these findings, highlighting the antimalarial capabilities of andrographolide extracted from sambiloto leaves. A study by Widyawaruyanti et al. (2014) used andrographolide extracted from dried sambiloto leaves and formulated it into tablets. In this study, an in vivo test was conducted on mice infected with *Plasmodium berghei* strain ANKA, a malaria-causing parasite. The andrographolide tablets were developed by combining two fractions of ethanol extract: Fraction A, obtained through fractionation with ethyl acetate, and Fraction B, purified from Fraction A. Despite both fractions containing diterpenoid lactones, their physical and chemical properties differed markedly. Fraction A, characterized by a dark green, sticky substance, appeared to diminish the active substance's efficacy and reduced bioavailability in vivo (31).

The tablets were prepared using two unique techniques: wet granulation and dry dispersion, resulting in three distinct phytopharmaceutical products. These included Tablet I (wet granulation of Fraction A), Tablet II (wet granulation of Fraction B), and Tablet III (dry dispersion of Fraction B) (31). The antimalarial activity of these tablets was evaluated using the Peter test, a 4-day suppressive assay, where the tablets were administered orally to infected mice at a dose of 12.55 mg andrographolide/kg suspended in 0.5% CMCNa solution. Mice receiving only 0.5% CMCNa served as negative controls.

On the fifth day, blood samples were taken from each mouse to determine parasitemia levels by counting the number of infected red blood cells among a thousand randomly selected cells under a microscope. The parasitemia inhibition was calculated by subtracting the proportion of infected red blood cells in the test samples from the negative control, with results expressed as a percentage of the inhibition.

The study by Widyawaruyanti et al. (2014) indicated that all three tablet formulations inhibited parasitic growth in the blood, albeit with varying degrees of effectiveness, as evidenced by different percentage inhibition rates. The dosage of andrographolide was consistent across all treatments at 25.10 mg/kg/day (31).

Table 3 Provides detailed observations of the antiplasmodial activity of Tablets I, II, and III, reflecting their varying 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	-

3.1. Additional Information

- Data are expressed as mean ± standard deviation for five mice per group with F = 53.789.
- P < 0.001; comparison with control.

3.2. Interpretations

- Tablets I, II, and III demonstrate significant inhibition of parasitemia compared to the control group, as indicated by the P-value of less than 0.001.
- Tablet I, Tablet II, and Tablet III show varying degrees of efficacy, as evidenced by their different mean parasitemia percentages. The statistical analysis confirms that the differences are significant.

This detailed table and additional information underline the effectiveness of andrographolide-containing tablets in inhibiting malaria parasitic growth, showcasing their potential as therapeutic options in malaria treatment.

Based on the data presented in Table 3 and analyzed using one-way ANOVA at a 95% confidence level, 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 was not statistically significant. These findings suggest that the primary factor influencing the antimalarial activity of the tablets is the concentration of the active substances they contain, rather than the method of tablet preparation. Specifically, Tablet I, which is based on a less purified extract, demonstrates more pronounced antimalarial effects compared to Tablets II and III. Tablets II and III use andrografolide extracted from a more purified source (Extract AP Fraksi B), which enhances its concentration and potency. This indicates that the purification process significantly improves the effectiveness of andrografolide as an antimalarial agent. Consequently, the higher concentration of active andrografolide in Tablets II and III results in greater antimalarial efficacy, highlighting the importance of extract purification in maximizing the therapeutic potential of the tablets (31).

4. Conclusion

The sambiloto plant, although not yet widely recognized, has demonstrated significant potential in in vitro and in vivo studies as an alternative treatment for malaria, comparable to chloroquine from the cinchona plant. The leaves or herbs of sambiloto are capable of inhibiting heme polymerization in Plasmodium parasites, particularly Plasmodium falciparum, the primary cause of tropical malaria, with high efficacy because it has not yet been affected by genetic resistance factors. The compound andrografolide, found in the leaves or herbs of sambiloto, offers various therapeutic benefits. In addition to acting as an antioxidant that protects the body from oxidative damage, andrografolide also functions as an antimicrobial in several vital organs, as an antitumor agent in early-stage breast cancer, and as a hepatoprotector that safeguards the liver. Therefore, the extraction of andrografolide must be carried out using appropriate techniques that follow its physical and chemical characteristics to ensure that the resulting extract is effective in enhancing antimalarial mechanisms. The most effective extract for treating malaria is one with a high concentration of andrografolide. This can be achieved through purification methods that remove unwanted compounds, resulting in an optimal composition of andrografolide. Thus, the extract can provide maximum pharmacological effects with minimal risk of toxicity and undesirable side effects.

Further research in clinical trials is necessary to ensure the procedures and safety of sambiloto leaf extract when consumed by humans, as well as its effectiveness within the human body. Testing on individuals with simple complexities only provides a basic reference framework, but additional studies are required to validate its comprehensive efficacy and safety in more complex and diverse human populations.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared no conflict of interests.

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