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Antimalarial Activity of Ugonin J and Ugonin K Isolated from Tunjuk Langit (*Helminthostachys zeylanica*)

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Abstract. Malaria is a life-threatening disease caused by Plasmodium parasites, transmitted by Anopheles mosquitoes, and remains a significant global health issue. This study aimed to identify the antimalarial activity of ugonin J and ugonin K isolated from tunjuk langit (*Helminthostachys zeylanica*). The in vitro antimalarial assay was conducted using the 3D7 strain of Plasmodium falciparum, which is sensitive to chloroquine, with ugonin J and ugonin K concentrations ranging from 0.01 to 100 µg/mL. The parasitemia percentage was assessed at 48 hours post-treatment, and the percentage inhibition was calculated. The results showed a significant dose-dependent inhibition, with an IC_{50} value of 0, 041 µg/mL and 0.12 µg/mL, indicating potent antimalarial activity. Ugonin J and ugonin K exhibited effective inhibition of parasitemia at concentrations as low as 1 µg/mL, supporting its potential as a promising antimalarial agent. This study suggests that *Helminthostachys zeylanica* could be a valuable source of antimalarial compounds.

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1. Introduction

Malaria is one of the most threatening diseases with a high mortality rate. This disease can occur in both developed and developing countries. According to data from the WHO, nearly half of the global population living in malaria-endemic areas is at risk, resulting in 627,000 deaths in 2020 and infecting approximately 247 million people in 85 endemic countries, which led to 619,000 deaths in 2021 and in 2022, it was estimated that there were around 249 million cases of malaria globally (World Health Organization, 2021). In Indonesia, malaria cases are still quite high where the number of cases in 2018 was 46,987 cases (Khairuddin et al., 2023). Malaria is an infectious disease caused by the plasmodium parasite transmitted from the bite of *Anopheles sp* mosquitoes with symptoms of continuous chills and fever (Utami & Febrianti, 2022).

Efforts to eradicate malaria have been hindered due to the development of *Plasmodium falciparum* resistance to antimalarial drugs. This is caused by genetic mutations in the parasite that naturally allow them to survive despite being exposed to the medications (Latifah et al., 2020). Numerous studies have explored the benefits of natural substances derived from plants, both in the form of extracts and pure compounds isolated from plants. In Indonesia, malaria treatment until now still uses *athermisnin* or ACT (*Atermisnin Combination Therapy*) with an average of 78.83%, which shows that resistance is still a problem that affects the success of malaria cure (Kemenkes RI, 2023). As is well known, Indonesia is a country rich in medicinal plants, which have been utilized for centuries in traditional medicine. One of these plants is tunjuk langit or *Helminthostachys zeylanica.* This fern has been widely used by local communities for its medicinal properties. The Tunjuk Langit plant is also rich in humus and organic matter and can thrive in lowland areas with moist soil conditions. Phytochemical analyses have revealed that it contains saponins, steroids, flavonoids, and phenolics (Fitrya & Muharni, 2013), which are known for their pharmacological activities.

The plant Tunjuk Langit, scientifically known as *Helminthostachys zeylanica L.* Hook., is a type of fern that has long been utilized by the community as traditional medicine, providing various benefits to humans. H. zeylanica is one of the plants found in Riau Province and is traditionally used for medicinal purposes (F. El Ridhasya et al., 2020). In Indonesia, *H. zeylanica* is used by the community as a traditional medicine for treating ailments such as dysentery, colds, early-stage tuberculosis, and, when the leaves are dried, it can be used as a remedy for nosebleeds. Additionally, the young leaves of this plant can be consumed as vegetables (Ginantra et al., 2015)

H. zeylanica contains saponins, flavonoids, stilbenes and phenolics which have antioxidant (Huang et al., 2003), anti-inflammatory (Huang et al., 2017), anti-osteoporotic (Huang et al., 2017), antihyperuricemic and antidiabetic activities(F. El Ridhasya et al., 2020). Chalcone is a flavonoid compound that has known activity to inhibit parasite growth through the mechanism of inhibiting the cysteine protease enzyme in parasites (Khairuddin et al., 2023). Based on the research that has been done, the methanol extract of A. camansi Blanko stem wood has IC_{50} = 1.84 µg/ml which based on phytochemical tests shows the presence of flavonoids, terpenoids and tannins (Hakim, Aliefman., 2010). Among the bioactive compounds, ugonin—a group of flavonoids—has been identified as a major component of *Helminthostachys zeylanica.* One particular compound, ugonin J and ugonin K, has shown diverse biological potentials. Previous studies have reported its anti-inflammatory activity through the inhibition of osteoclast differentiation (Huang et al., 2017). Moreover, ugonin K demonstrates antidiabetic properties (F. El Ridhasya et al., 2020) and acts as a strong antioxidant (Lee et al., 2012). Ugonin J also has several bioactivities such as antidiabetic with IC_{50} = 273.13 ppm (F. E. Ridhasya et al., 2019) and antioxidant (Huang et al., 2003).

These promising bioactivities highlight ugonin K as a potential candidate for therapeutic development, including as an antimalarial agent. Therefore, this study aims to investigate and evaluate the antimalarial activity of ugonin K isolated from *Helminthostachys zeylanica.*

2. The Methods

2.1. Sample preparation

1 mg of ugonin J and ugonin K is dissolved in 100 µL of DMSO (stock concentration: 10,000 µg/mL). This solution is further diluted to achieve the appropriate concentrations for biological testing.

2.2. Parasite preparation

The parasites used are synchronized to the ring stage with a parasitemia of approximately 1%. This ensures that all parasites are at the same phase, enhancing the accuracy of the results.

2.3. In vitro antimalarial activity assay

2 µL of the test solution at various concentrations (100, 10, 1, 0.1, 0.01 µg/mL) is mixed with 198 µL of the parasite solution. The total volume per well is 200 µL. The concentrations of the compound are tested in duplicate (R1 and R2) to improve reliability. The culture is incubated in a chamber with a gas mixture of 5% CO_2, 5% O_2, and 90% N_2 for 48 hours at 37°C.

2.4. Data analysis

Data processing using Graphpad Prism 10 for macOS.

3. Result and Discussion

Based on a source, the antiplamodial activity is categorized as excellent $IC_{50} \leq 5$ µg/ml, good $5 < IC_{50} \leq 10$ µg/ml, medium $10 < IC_{50} \leq 20$ µg/ml, low $20 < IC_{50} \leq 40$ µg/ml and inactive $IC_{50} > 40$ µg/ml (Muganza et al., 2016). This table shows the effect of ugonin J and ugonin K concentration on *Plasmodium falciparum parasitemia* with the percentage inhibition at various concentrations (0.01 µg/mL to 100 µg/mL). Antimalarial activity is measured based on the inhibition of *parasitemia* after 48 hours of incubation.

Table 1. Inhibitory effect of ugonin K on *Plasmodium falciparum*

Concentration (µg/mL)	R	%Parasitemia		% Growth	% Inhibition	%Avarege Inhibition
		0 hours	48 hours			
Control (-)	1	0.87	10	9.13	-	-
	2	0.87	10.6	9.19	-	
0.01	1	0.87	7.99	7.12	22.02	22.60%
	2	0.87	7.93	7.06	23.18	
0.1	1	0.87	5.59	4.72	48.30	48.96%
	2	0.87	5.50	4.63	49.62	
1	1	0.87	3.31	2.44	73.27	72.98%
	2	0.87	3.38	2.51	72.69	
10	1	0.87	1.84	0.97	89.38	89.63%
	2	0.87	1.80	0.93	89.88	
100	1	0.87	0	0	100	100
	2	0.87	0	0	100	

Based on the data obtained, the biological activity assay of ugonin K demonstrated significant effectiveness as an antimalarial compound. The assay was conducted across a concentration range of 0.01–100 µg/mL. At the highest concentration of 100 µg/mL, ugonin K completely inhibited parasitemia, with an inhibition percentage of 100%. This indicates that at high concentrations, ugonin K is capable of fully inhibiting the development of parasites. At a concentration of 10 µg/mL, the compound showed an average inhibition of 89.63%, indicating very high effectiveness. Even at lower concentrations, such as 1 µg/mL and 0.1 µg/mL, ugonin K still exhibited good inhibitory activity, with average inhibition percentages of 72.98% and 48.96%, respectively. This suggests that ugonin K remains effective in inhibiting parasitemia even at lower concentrations. However, at a concentration of 0.01 µg/mL, the recorded 22.60%, indicating a decrease in effectiveness at very low concentrations.

Table 2 shows the effect of ugonin J concentration on *Plasmodium falciparum parasitemia* with the percentage inhibition at various concentrations (0.01 µg/mL to 100 µg/mL). Antimalarial activity is measured based on the inhibition of *parasitemia* after 48 hours of incubation.

Table 2. Inhibitory effect of ugonin J on *Plasmodium falciparum*

Concentration (µg/mL)	R	%Parasitemia		% Growth	% Inhibition	%Avarege Inhibition
		0 hours	48 hours			
Control (-)	1	0.87	10	9.13	-	-
	2	0.87	10.06	9.13	-	
0.01	1	0.87	6.89	6.02	34.06	34.39
	2	0.87	6.87	6.00	34.71	
0.1	1	0.87	4.80	3.93	56.96	56.88
	2	0.87	4.84	3.97	56.80	
1	1	0.87	1.96	1.09	88.06	88.15
	2	0.87	1.95	1.08	88.25	
10	1	0.87	0.65	0	100	100
	2	0.87	0.69	0	100	
100	1	0.87	0	0	100	100
	2	0.87	0	0	100	

Based on Table 2, it can be seen that the activity of ugonin J shows significant effectiveness as an antimalarial compound. The concentration range tested was the same as ugonin K, namely 0.01-100 / ml. At concentrations of 10 and 100 / ml, ugonin J inhibited parasitemia completely with a percentage of 100%. From this experiment, it shows that at high concentrations ugonin J is able to inhibit parasite development completely. At concentrations of 0.1 and 1 / ml, ugonin J showed good inhibitory activity with percentages of 56.88% and 88.15% respectively. This shows that ugonin J is still effective in inhibiting parasitemia even at lower concentrations. At a concentration of 0.01, it was recorded at 34.39%, which shows that at this concentration the inhibitory effectiveness is lower.

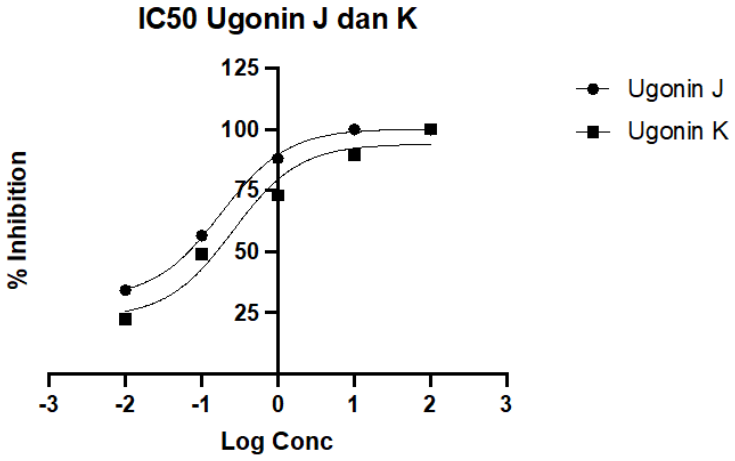

Figure 1. IC_{50} ugonin J and ugonin K.

Based on the results of the log curve analysis (inhibitor) vs response using Graphpad Prism as shown in Figure 1, the IC_{50} value for ugonin J was 0.1790 and ugonin K had an IC_{50} value of 0.2571. The IC_{50} value of ugonin J is smaller than ugonin K. This shows that the inhibitory potential of ugonin J is stronger and has antifungal activity. Ugonin J is stronger and has higher antifungal activity than ugonin K. The R^2 value (coefficient of determination) of each curve where ugonin J shows an R^2 value = 0.9987 which shows very good data suitability to the sigmoid model. Meanwhile, ugonin K has an R^2 value = 0.9658 which is still in the good category.

4. Conclusion

Based on the results of in vitro tests, ugonin K and ugonin J from *Helminthostachys zeylanica* showed strong antimalarial activity. Its effectiveness was high at concentrations of 10–100 µg/mL, indicating significant inhibition of parasitemia. The low IC_{50} values, namely 0.12 µg/mL and 0.041 µg/mL, indicated that ugonin K and ugonin J have very good potential as antimalarial compounds. The lower the IC_{50} value, the higher the potential of the compound as an antimalarial agent. At lower concentrations (0.01 µg/mL), its effectiveness decreased but still showed a typical dose-response relationship. In data analysis using Graphpad Prism 10 for macOS, the IC_{50} of ugonin J was smaller than ugonin K, namely 0.1790 and 0.2571. Therefore, ugonin J and ugonin K can be promising candidates for the development of antimalarial drugs. Where ugonin J has a stronger inhibitory potential than ugonin K.

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