

Synergism of Anti-Malarial Effect of *Carica papaya* L. and *Moringa oleifera* Leaf Extract in Mice

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ABSTRACT

Malaria parasites, *Plasmodium spp*, can develop resistance to anti-malarial drugs. Hence, potential alternative therapeutic agents should be explored. This research aimed to investigate the anti-malarial effect of a combination of *Carica papaya* and *Moringa oleifera* leaf extract. Peter's test performed on Balb/c mice to investigate the malarial parasite growth inhibition. Mice were infected with *Plasmodium berghei* by intraperitoneal injection to provoke rodent malaria. After infection, we grouped every 5-6 mice into different treatment groups, including a negative control group, a positive control group treated with artesunate, a moringa group, a papaya group, and a combination therapy group. Treatments were initiated 3 hours after infection (day 0) and continued every day until day 3. On day 4, we examined a thin smear of the tail vein blood for parasitemia to calculate the suppression rate. The result shows that the parasite suppression rate of *C. papaya* leaf extract (CPLE) is 15.02 % (percent parasitemia 4.56±1.96), the suppression rate of *M. oleifera* leaf extract (MOLE) is 17.32% (percent parasitemia 4.44±1.78) and that of the combination extract (CE) is 28.73% (percent parasitemia 3.82±1.48). In conclusion, there is a synergism of the anti-malarial effect of the combined leaf extract of *C. papaya* and *M. oleifera*.

Keywords: Anti-malarial drugs; *Carica papaya* L.; *Moringa oleifera*; Parasite suppression; Peter's test; *Plasmodium berghei*

INTRODUCTION

Malaria is transmitted by the Anopheles mosquito vector, which carries the *Plasmodium spp.* parasite in its salivary glands and injects the parasites into the human body during the blood meal (CDC, 2020; Yusuf et al., 2019). Malaria is still a life-threatening disease, causing a high number of deaths, especially among children in endemic areas in Sub-Saharan Africa (WHO, 2021). As reported in the WHO World Malaria Report 2020, several regions in Indonesia are malaria endemic, with the total number of malaria cases ranked second in Southeast Asia. Most cases occurred in Papua Province (86%), with 216,380 cases in 2019, followed by East Nusa Tenggara (NTT) and West Papua (Kemenkes RI, 2021)

Artemisinin-based combination therapy (ACT) is the first-line drug for uncomplicated

malaria since 2004 due to its greater efficacy in treating the disease, reducing transmission in endemic areas, and lowering rates of malaria reinfection (Pousibet-Puerto et al., 2016). The use of ACT also reduces the length of hospital stay, increases the parasite clearance rate, and reduces disease mortality. Artemisinin is produced from the extract of the *Artemisia annua* plant originating from China (Haq et al., 2020). However, reports indicate the emergence of artemisinin-resistant strains, reducing efficacy in malaria treatment (Phyo et al., 2012). Therefore, it is necessary to search for alternative medicinal compounds that can inhibit the growth of malaria parasites.

Indonesia is rich in natural resources, including various plants used as traditional medicine. Some medicinal plants in Indonesia that have been extracted and tested for their anti-malarial effects are papaya, bitter, cocoa bean, clove flower, and Moringa (Rehena, 2010; Veronica et al., 2020; Septiana et al., 2017;

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Faisal et al., 2013). Among these plants, papaya (*C. papaya* L.) and Moringa (*M. oleifera*) are easy to obtain because locals in Makassar, South Sulawesi, cultivate them. Studies have reported that papaya leaf extract inhibits *Plasmodium falciparum*'s growth *in vitro* (Rehena, 2010) and *Plasmodium berghei* *in vivo* (Indah, 2010). The crude papaya leaf ethyl acetate isolate has an excellent anti-plasmodial effect against *P. falciparum* and resistant strains of *P. falciparum* (Melariri, 2011). *C. papaya* leaf extract also enhanced the anti-malarial effect of artesunate (Oraebosi & Good, 2021). While the seeds of the Moringa plant have been widely used as an alternative anti-malarial treatment in several African countries with low toxicity (Hermanto et al., 2013; Obediah & Obi, 2020), a study reported that the Moringa leaf extract has an inhibitory effect on the growth of *P. berghei* when combined with Artemisinin (Veronica et al., 2020; Somsak et al., 2018). These two extract compounds are thought to have similar flavonoids, such as quercetin and kaempferol, that have antioxidant effects against *Plasmodium* (Melariri, 2011; Bezerra, 2023). However, the specific mechanism of these two extracts is still unclear.

There may be a synergistic effect of both extracts. A synergistic effect is an increased parasite suppression rate when two drugs are used together rather than a single drug (Oraebosi & Good, 2021; Rasoanaivo et al., 2011). Combination therapy in treating malaria is essential to prevent the development of drug-resistant parasites. Therefore, we aimed to investigate the anti-malarial effect of administering the leaf extract of *C. papaya* in combination with the leaf extract of *M. oleifera*. We hypothesized that combination therapy has a better parasite suppression rate than single therapy.

MATERIALS AND METHODS

Materials

Chemicals and Animal Experimental

We obtained a cryopreserved stock of *P. berghei* strain ANKA from Prof. Laurent Renia at the Agency for Science, Technology, and Research (A*STAR) in Singapore with the permission of Professor Syafruddin from Eijkman Institute for Molecular Biology and Dr. Puji Setia Budi Asih from National Innovation and Research Agency (BRIN) to induce the *Plasmodium* infection. We purchased the pure artesunate powder from Shandong Natural Micron Pharm Tech. The artesunate was dissolved in 0.5% CMC to administer 6 mg/kg BW

to the control positive group. We obtained six to eight-week-old Balb/c mice weighing 20-30 g from Gold Mice Farm. The mice were acclimatized in the animal room for one week before the experiment. They were housed at a temperature of 25-28°C and provided with standard diet pellet AD I and drinking water. All experiments were approved by the Ethical Committee of the Faculty of Medicine at Hasanuddin University (Document number: 764/UN4.6.4.5.31/PP36/2022).

Plant Materials

We obtained local variants of *C. papaya* and *M. oleifera* from Kebun Edukasi Kelor (Moringa Education Garden) in Makassar, South Sulawesi province. The Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, identified the plant materials, and the herbarium was deposited in the Department of Parasitology, Faculty of Medicine, Universitas Hasanuddin.

Methods

Preparation of Methanol Crude Extracts

The extraction of moringa leaves and papaya leaves was performed using maceration. Each 100 g of Moringa leaf and papaya leaf simplicia was extracted using 1L of 100% methanol (Pro analysis, Merck, Germany) (1:10). Maceration was carried out at room temperature (25°C) for 5 days with daily stirring. Filtration was carried out using Whatman filter paper (GE Healthcare Life Sciences, size 41, diameter 110 mm) on a Buchner funnel (diameter 150 mm) using a vacuum pump (airflow capabilities 0,65 L/min; vacuum 29 Hg). Solvent evaporation was done using a rotary evaporator (Heidolph G3, Schawabach, Germany) at 60°C with a rotation speed of 80 rpm until a thick extract was obtained. The evaporation of the solvent was continued in the water bath. Remaceration was performed using the same method. All extracts obtained were weighed to obtain a percentage yield.

Anti-malarial activity test

The extract's efficacy was evaluated using Peter's 4-day suppressive test by injecting the mice with 1×10^7 infected red blood cells intraperitoneally from the donor mice. The parasite stock was injected intraperitoneally into Balb/c mice as the donor. Parasitemia was determined by microscopy of Giemsa (Sigma-Aldrich) stained blood smear. A drop of tail vein blood was taken to make the thin blood smear and then fixed with methanol (Pro analysis, Merck, Germany) before staining with Giemsa 3% for

30 minutes. Parasitemia was calculated as a percentage of infected erythrocytes per one thousand erythrocytes (Fentahun S et al., 2016).

The mice were then divided into several groups, including the negative control group, positive control group treated with artesunate, *M. oleifera* group, *C. papaya* group, and the combination therapy group (Table I).

The extract was administered orally 3 hours after parasite injection on day 0 to the treatment groups. The solvent was administered to negative control mice whereas 6 mg/kg BW artesunate was administered to positive control mice. The treatment was continued once daily every 24 hours for three days (days 1-3). On day 4, a drop of tail vein blood was taken to make a thin blood smear for parasitemia analysis. The formula for calculating the percentage of suppression is as follows (Philipsson, 1991)

$$\text{parasite suppression (\%)} = \frac{P_n - P_t}{P_n} \times 100$$

Notes: P_n = parasitemia of the negative control group (average); P_t = parasitemia of the treated group (average)

Data analysis

Graphpad prism® was used to analyze this study. Parasitemia results were expressed as mean \pm standard error of the mean (SEM). An unpaired *t*-test was used to compare the two groups, and significant differences were considered at 95% confidence, $P < 0.05$

RESULTS

After a consecutive four-day treatment since parasite injection, the blood smears of the mice were evaluated for parasitemia. The average day-4-parasitemia rate was compared between the treated and negative control groups (Table II). From table II, it is shown that the average of parasitemia in mice given a combination of therapy was lower than those given either CPLE or MOLE only (3.82 ± 1.48 VS 4.56 ± 1.96 and 4.44 ± 1.78). Even though the differences in parasitemia between the combination group and CPLE only or MOLE only were not statistically significant ($p=0.7725$ and $p=0.8320$, respectively).

Although the parasitemia differences were statistically insignificant, the parasite suppression rate of the combined extract was almost double that of the single extract (Figure 1). The percentage of parasite suppression of CPLE was 15.02 %, whereas MOLE achieved a 17.32 % suppression rate. The suppression rate achieved by combination therapy was 28.74 %. The parasite suppression rate of the combined extract was

almost double that of the single extract (Figure 1). An enhancement of effect by combining two drugs means a synergistic effect (Oraebosi & Good, 2021). Therefore, our result infers that there may be synergistic actions of active compounds in MOLE and CPLE.

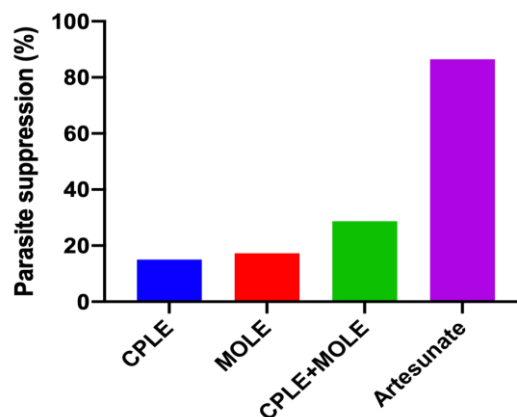


Figure 1. Suppression rate of *Plasmodium berghei* in mice. Mice ($n=4-5$) were infected with 10^7 iRBC and then administered with the extract or artesunate. The average parasitemia of the treatment group was compared with the solvent group to calculate the suppression rate. CPLE: *C. papaya* leaf extract; MOLE: *M. oleifera* leaf extract.

The parasite suppression rate of the artesunate was 86.49% (Figure 1). Compared with the artesunate, the parasitemia rate of the mice given the combination therapy was much higher (3.82 vs 0.73 but not significantly different ($p=0.1049$)) (Table II).

DISCUSSION

In this study, 200 mg/kgBW of CPLE and 200 mg/kgBW of MOLE demonstrated a higher parasite suppression than the single extract administration. A study reported that the average parasitemia of mice treated with 350 mg/kg BW of CPLE on day 3 was around 25 % (Okpe et al., 2016), which was much higher than the parasitemia in the current study. On the other hand, Harapan et al. (2015) reported that the average percent parasitemia in mice given 3.75 mg of MOLE was about 3.8 % on day 3. This dose may reduce the inflammation in cerebral malaria by decreasing the expression of NF- κ B.

An enhancement of effect by combination of two drugs means a synergistic effect (Oraebosi & Good, 2021). Here, our result demonstrated that there may be synergistic actions of active compounds in MOLE and CPLE. Several compounds in MOLE that may have anti-malarial

Table I. Experimental group

Group	Treatment*	Dose
Negative control	CMC 0,5 %	100 uL
Positive control	artesunate	6 mg/kg BW
Papaya group	<i>C. papaya</i> leaf extract (CPLE)	200 mg/kg BW
Moringa group (MOLE)	<i>M. oleifera</i> leaf extract (MOLE)	200 mg/kg BW
Combination group	<i>C. papaya</i> leaf extract and <i>M. oleifera</i> leaf extract (CPLE+MOLE)	200 mg/kg BW ¹

*Each extract was given in 200 mg/kg BW

Table II. Average of parasitemia between groups

Group	% Parasitemia (mean \pm SEM)
CMC	5.37 \pm 1.72
CPLE	4.56 \pm 1.96
MOLE	4.44 \pm 1.78
CPLE+MOLE	3.82 \pm 1.48*
artesunate	0.73 \pm 0.08*

Notes: Mice ($n=4-5$) were infected with 10^7 iRBC and then administered with the extract or artesunate for four days. On day 4, the parasitemia rate was calculated. CMC: the solvent; CPLE: *C. papaya* leaf extract; MOLE: *M. oleifera* leaf extract; *not significantly different

properties are flavonoids, alkaloids, quercetin, and kaempferol (Lehane & Saliba, 2008; Ezenyi et al., 2014; Somsak et al., 2018), whereas in CPLE are alkaloids, carpaine, and flavonoid (Atanu et al., 2021; Sannella et al., 2019; Teng et al., 2019).

It is suggested that pharmacodynamic synergy could be achieved better by the combination of whole plant extract rather than the combination of single compounds of each plant (Rasoanaivo et al., 2011). In addition, studies have reported that combining artesunate with MOLE or artesunate with CPLE achieved better parasite suppression than artesunate only (Somsak et al., 2016; Oraebosi & Good, 2021). Therefore, we expected a better effect with two crude extracts. However, we did not achieve this expectation. The artesunate administration showed a much higher suppression rate compared with the combined leaf extract. We assume that optimization of doses may improve the result of the combined extract.

The anti-malarial properties of moringa leaves might be associated with several plant flavonoids, such as apigenin, kaempferol, rutin, and quercetin, that have been reported to have a potential for parasite inhibition. However, the exact mechanism remains unknown (Bezerra et al., 2023). It is reported that these two extracts have antioxidant and immunomodulatory properties derived from flavonoids, such as quercetin and kaempferol, which may improve any damages caused by free radical production due to Plasmodium infection (Melariri, 2011; Bezerra,

2023). In addition, several properties in papaya leaves, such as flavonoid, tannin, terpenoid, and polyphenol, might have enzyme inhibitory effects that inhibit some of the parasite's key enzymes (Oraebosi & Good, 2021). However, since these two extracts' specific mechanism of action has not been identified, further research is required to review the inhibitory mechanism of both extracts against Plasmodium specifically.

One limitation of this study is that only one dose of each extract, 200 mg/kgBW, was applied because several studies previously have reported that the dose of 200 mg/kg CPLE (Mulisa et al., 2018) and 100 mg/kg BB MOLE actively suppressed the parasite growth in mice (Dondee et al., 2016). However, chloroquine rather than artesunate was the standard drug used in both studies. Therefore, we need to evaluate several combinations of doses in future studies.

Despite the limitation of this study, we demonstrate that the combination of leaf extract of *M. oleifera* and *C. papaya* has the potential to be an alternative anti-malarial drug that is worth further investigation.

CONCLUSION

Although the percentage of malaria parasite suppression of the combination of *C. papaya* leaf extract and *M. oleifera* leaf extract is relatively low, there could be synergistic anti-malarial actions of their active compounds. It is necessary to evaluate the best combination of doses of the extracts.

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REFERENCES

- Atanu, F. O., Idih, F. M., Nwonuma, C. O., Hetta, H. F., Alamery, S., & El-Saber Batiha, G. (2021). Evaluation of antimalarial potential of extracts from *Alstonia boonei* and *Carica papaya* in plasmodium berghei-infected mice. *Evidence-Based Complementary and Alternative Medicine*, 2021, 1–11. <https://doi.org/10.1155/2021/2599191>
- Bezerra, J. J., Pinheiro, A. A., & Dourado, D. (2023). Antimalarial potential of *Moringa Oleifera* Lam. (Moringaceae): A review of the ethnomedicinal, pharmacological, toxicological, and phytochemical evidence. *Journal of Venomous Animals and Toxins Including Tropical Diseases*, 29. <https://doi.org/10.1590/1678-9199-jvatitd-2022-0079>
- Centers for Disease Control and Prevention. (2020, October 6). CDC - dpdx - malaria. Centers for Disease Control and Prevention. <https://www.cdc.gov/dpdx/malaria/index.html>
- Dondee, K., Bootprom, P., Saiphet, B., Borkaew, P., Klubsri, C., & Somsak, V. (2016). Activities of *Moringa Oleifera* Leaf Extract Against Plasmodium Berghei ANKA Infection in ICR Mice. *International Journal of Innovative Research in Medical Science*, 1(6), 194–201.
- Ezenyi, I. C., Salawu, O. A., Kulkarni, R., & Emeje, M. (2014). Antiplasmodial activity-aided isolation and identification of quercetin-4'-methyl ether in *Chromolaena odorata* leaf fraction with high activity against chloroquine-resistant Plasmodium falciparum. *Parasitology Research*, 113(12), 4415–4422. <https://doi.org/10.1007/s00436-014-4119-y>
- Fentahun, S., Makonnen, E., Awas, T., & Giday, M. (2017). In vivo antimalarial activity of crude extracts and solvent fractions of leaves of *Strychnos mitis* in plasmodium berghei infected mice. *BMC Complementary and Alternative Medicine*, 17(1). <https://doi.org/10.1186/s12906-016-1529-7>
- Haq, F. U., Roman, M., Ahmad, K., Rahman, S. U., Shah, S. M., Suleman, N., Ullah, S., Ahmad, I., & Ullah, W. (2020). Artemisia annua: Trials are needed for COVID-19. *Phytotherapy Research*, 34(10), 2423–2424. <https://doi.org/10.1002/ptr.6733>
- Harahap, H. S., Dalhar, M., H, T. E., Norahmawati, E., & Fitri, L. E. (2015). Kombinasi artemisinin Dan Ekstrak moringa Oleifera Menurunkan ekspresi NF-Kb namun tidak menurunkan Ekspresi Inos Pada Otak mencit Diinfeksi malaria. *Jurnal Kedokteran Brawijaya*, 28(3), 195–201. <https://doi.org/10.21776/ub.jkb.2015.028.03.6>
- Hermanto, F., Iwo, M. I., & Asih, P. (2013). Effect of ethanolic extract of clove (*Eugenia caryophyllata* Thunb) as anti-malaria activity on Plasmodium falciparum. *Jurnal Tumbuhan Obat Indonesia*, 6(2).
- Hermanto, F., Yun, Y. F., Aisyah, L. S., Saputra, T. R., Hakim, A. R., Ningsih, A. K., Herlina, T., Julaha, E., Zainuddin, A., & Supratman, U. (2014). Uji Aktivitas Antimalaria Ekstrak etanol Daun Cocor Bebek (*Kalanchoe Blossfeldiana* poelln.) pada plasmodium falciparum 3D7. *Kartika Jurnal Ilmiah Farmasi*, 2(2). <https://doi.org/10.26874/kjif.v2i2.18>
- Lehane, A. M., & Saliba, K. J. (2008). Common dietary flavonoids inhibit the growth of the intraerythrocytic malaria parasite. *BMC Research Notes*, 1(1), 26. <https://doi.org/10.1186/1756-0500-1-26>
- Indah, R. (2014). Uji aktivitas anti malaria ekstrak air daun pepaya gantung (*Carica papaya* L pada mencit yang diinfeksi Plasmodium berghei (thesis). Perpustakaan Universitas Negeri Jember, Jember.
- Melariri, P., Campbell, W., Etusim, P., & Smith, P. (2011). Antiplasmodial properties and bioassay-guided fractionation of ethyl acetate extracts from carica papayaleaves. *Journal of Parasitology Research*, 2011, 1–7. <https://doi.org/10.1155/2011/104954>
- Mulisa, E., Girma, B., Tesema, S., Yohannes, M.,

- Zemene, E., & Amelo, W. (2018). Evaluation of in vivo antimalarial activities of leaves of moringa oleifera against plasmodium berghei in mice. *Jundishapur Journal of Natural Pharmaceutical Products*, In Press(In Press). <https://doi.org/10.5812/jjnpp.60426>
- Okpe, O., Habila, N., Ikwebe, J., Upev, V. A., Okoduwa, S. I., & Isaac, O. T. (2016). Antimalarial potential of carica papaya and vernonia amygdalina in mice infected with plasmodium berghei. *Journal of Tropical Medicine*, 2016, 1–6. <https://doi.org/10.1155/2016/8738972>
- Obediah, G. A., & Christian Obi, N. (2020). Anti-plasmodial effect of moringa oleifera seeds in plasmodium berghei infected albino rats. *Biochemistry & Pharmacology: Open Access*, 09(01). <https://doi.org/10.35248/2167-0501.20.9.268>
- Oraebosi, M. I., & Good, G. M. (2021). Carica papaya augments anti-malarial efficacy of artesunate in Plasmodium berghei parasitized mice. *Annals of Parasitology*, 67(2), 295–303.
- Phillipson, J. D. (1991). Assays for antimalarial and amoebicidal activities. Academic Press Inc. (London) Ltd.
- Phyo, A. P., Nkhoma, S., Stepniewska, K., Ashley, E. A., Nair, S., McGready, R., ler Moo, C., Al-Saai, S., Dondorp, A. M., Lwin, K. M., Singhasivanon, P., Day, N. P., White, N. J., Anderson, T. J., & Nosten, F. (2012). Emergence of artemisinin-resistant malaria on the western border of Thailand: A longitudinal study. *The Lancet*, 379(9830), 1960–1966. [https://doi.org/10.1016/s0140-6736\(12\)60484-x](https://doi.org/10.1016/s0140-6736(12)60484-x)
- Pousibet-Puerto, J., Salas-Coronas, J., Sánchez-Crespo, A., Molina-Arrebola, M. A., Soriano-Pérez, M. J., Giménez-López, M. J., Vázquez-Villegas, J., & Cabezas-Fernández, M. T. (2016). Impact of using artemisinin-based combination therapy (ACT) in the treatment of uncomplicated malaria from Plasmodium falciparum in a non-endemic zone. *Malaria Journal*, 15(1). <https://doi.org/10.1186/s12936-016-1408-1>
- Rasoanaivo, P., Wright, C. W., Willcox, M. L., & Gilbert, B. (2011). Whole plant extracts versus single compounds for the treatment of malaria: Synergy and positive interactions. *Malaria Journal*, 10(S1). <https://doi.org/10.1186/1475-2875-10-s1-s4>
- Rehena, J. F. (2010). In Vitro Acitivities Test of Papaya Leaf Extract (Carica papaya. LINN) as an Antimalaria Agent. *Jurnal Ilmu Dasar*, 11, 96–100.
- RI, K. (2021). Wilayah-Wilayah Endemis Malaria tinggi di Indonesia. *Wilayah-wilayah Endemis Malaria Tinggi di Indonesia | Malaria.id*. <https://malaria.id/artikel/wilayah-wilayah-endemis-malaria-tinggi-di-indonesia>
- Sannella, A. R., Karioti, A., Orsini, S., Scalone, A., Gradoni, L., Messori, L., Severini, C., & Bilia, A. R. (2019). Leaf decoction of Carica papaya combined with artesunate prevents recrudescence in plasmodium berghei-infected mice. *Planta Medica*, 85(11/12), 934–940. <https://doi.org/10.1055/a-0952-3677>
- Septiana, E., Gianni, D., & Partomuan, S. (2017). Toxicity and In Vitro anti-malarial Heme Polymerization Inhibition Activity of Sambiloto (Andrographis paniculata) Leaf Extracts. *Media Penelitian Dan Pengembangan Kesehatan*, 27(4), 255–262.
- Somsak, V., Borkaew, P., Klubsri, C., Dondee, K., Bootprom, P., & Saiphet, B. (2016). Antimalarial properties of aqueous crude extracts of gynostemma pentaphyllum and moringa oleifera leaves in combination with artesunate in plasmodium berghei-infected mice. *Journal of Tropical Medicine*, 2016, 1–6. <https://doi.org/10.1155/2016/8031392>
- Somsak, V., Damkaew, A., & Onrak, P. (2018). Antimalarial activity of Kaempferol and its combination with chloroquine in plasmodium berghei infection in mice. *Journal of Pathogens*, 2018, 1–7. <https://doi.org/10.1155/2018/3912090>
- Teng, W.-C., Chan, W., Suwanarusk, R., Ong, A., Ho, H.-K., Russell, B., Rénia, L., & Koh, H.-L. (2019b). In vitro antimalarial evaluations and cytotoxicity investigations of carica papaya leaves and Carpaine. *Natural Product Communications*, 14(1). <https://doi.org/10.1177/1934578x1901400110>
- Veronica, E., Amelia, I., Yunatan, K. A., Christmayanti, N. K., & Mahendra, A. N. (2020). Potensi Kombinasi Ekstrak Daun Kelor (moringa oliefera) Dan Artemisia (artemisia annua) Sebagai antimalaria plasmodium falciparum. *Jurnal Ilmiah Kesehatan Sandi Husada*, 12(2), 831–841.

<https://doi.org/10.35816/jiskh.v12i2.415>
World Health Organization. (2021). Fact sheet about malaria. World Health Organization. <https://www.who.int/news-room/fact-sheets/detail/malaria>
Yusuf, Y., Yoshii, T., Iyori, M., Mizukami, H., Fukumoto, S., Yamamoto, D. S., Emran, T. B.,

Amelia, F., Islam, A., Syafira, I., & Yoshida, S. (2019). A viral-vectored multi-stage malaria vaccine regimen with protective and transmission-blocking efficacies. *Frontiers in Immunology*, 10. <https://doi.org/10.3389/fimmu.2019.02412>