RJC

RASĀYAN *J. Chem.*
Vol. 17 | No. 4 |2017-2024| October - December | 2024
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

CHEMICALLY ACTIVE COMPOUNDS AND ANTI-MALARIAL EFFICACY OF (*Syzygium cumini)* STEM EXTRACTS ORIGINATED FROM IE SEUM GEOTHERMAL AREA

M. Munira[1,✉], N. Zakiah[1] and M. Nasir[2]
[1]Department of Pharmacy, Aceh Health Polytechnics, Banda Aceh, (Aceh) Indonesia
[2]Department of Biology, Faculty of Mathematics and Natural Science, Universitas Syiah Kuala, Kopelma Darussalam, Banda Aceh, (Aceh) Indonesia
✉Corresponding author: munira.bio@poltekkesaceh.ac.id

ABSTRACT

In this study, we determined chemically active compounds extracted from Sy*zygium cumini* stem using four different solvents, ethyl acetate, n-hexane, ethanol, and methanol. Gas Chromatography-Mass Spectrometry was used to investigate the chemical constituents in the targeted plant material and the in vitro antimalarial assay was evaluated through Giemsa staining microscopy utilizing the Plasmodium falciparum strain 3D7. The results indicate that the number of active compounds in the extract of ethyl acetate, n-hexane, ethanol, and methanol is 23, 7, 5, and 21 respectively, there are more compounds found in the ethyl acetate and methanol compared to ethanol and n-hexane. The antimalarial test confirmed that all four extracts were able to stop the growth of tested plasmodium but the ethyl acetate extract showed a remarkable ability to prevent the growth of plasmodium falciparum and completely inhibit it at the concentration of 100 µg/mL with the lowest IC_{50} of 11.08 µg/mL. The finding of this study could be useful for further development of natural product-based drugs for malaria disease treatment.

Keywords: *Syzygium cumini,* Ethanolic Extract, Antimalarial Activity, Active Compounds, Geothermal Area.
RASĀYAN *J. Chem.*, Vol. 17, No.4, 2024

INTRODUCTION

Syzygium cumini, commonly known as the Java plum or black plum, has been extensively utilized in traditional medicine across various cultures for its wide range of therapeutic properties. [1] The plant is known for its rich phytochemical composition, which includes flavonoids, phenolic acids, tannins, and essential oils, making it a subject of interest for pharmacological studies. [2] Previous studies regarding the antimalarial activity of *S.cumini* have been reported by Maslachah and Purwitasari [3] who used the fruit of *S.cumini* extracted using different solvents. Maslachah and Sugihartuti (2018)[4] have also reported the potential of *S.cumini* leaf extracts as an antimalarial agent. Among the different parts of the plant, the stem has been identified as a valuable source of bioactive compounds with potential health benefits. Recent studies have highlighted the importance of these compounds in treating ailments such as diabetes, inflammation, and microbial infections. However, there is limited research on the detailed chemical profile of the stem and its potential antimalarial properties[5] To fill this gap, our research employed the maceration technique to extract bioactive compounds from the stem of *S. cumini* using four different solvents: ethyl acetate, n-hexane, ethanol, and methanol. The choice of these solvents was based on their varying polarity, which enables the extraction of a broad spectrum of chemical constituents. [6] Ethyl acetate, with moderate polarity, is effective in extracting both polar and non-polar compounds. [7,8]n-Hexane, being non-polar, is suitable for lipophilic substances. Ethanol and methanol, both polar solvents, vary slightly in their extraction efficiency due to differences in their polarity and boiling points[9,10]. The extracted compounds were then analyzed using Gas Chromatography-Mass Spectrometry (GC-MS), a robust analytical technique that allows for the precise identification and quantification of volatile and semi-volatile compounds[11] In addition to the chemical analysis, the extracts were subjected to antimalarial testing to evaluate their efficacy against malaria, a disease that continues to pose significant health challenges globally[12,13]

The integration of phytochemical profiling with biological testing aims to identify potential new antimalarial agents and contribute to the broader understanding of the medicinal value of *S. cumini*. This

http://doi.org/10.31788/RJC.2024.1748996

research not only pursues to expand the knowledge of the chemical constituents of *S. cumini* stem but also to explore its therapeutic potential, particularly in the context of malaria treatment.

EXPERIMENTAL

Material and Methods

The Ethyl acetate, n-hexane, ethanol, and methanol were purchased from Sigma-Aldrich, Singapore. DMSO and gas mixture were obtained from the tropical disease diagnostic center, Airlangga University, Surabaya, Indonesia.

Plant Material Collection

The stem of *S. cumini* was collected from the Ie Seum geothermal area, district of Aceh Besar, Aceh province, Indonesia, and was identified in the herbal laboratory of Biology, Faculty of Natural Sciences, Universitas Syiah Kuala, Banda Aceh.

Extraction

Extraction was conducted according to Raji *et al.,* 2019[14] Briefly, the collected *S. cumini* stem was thoroughly washed with distilled water to remove the dirt, and subsequently chopped and airdried for 3 days to evaporate the moisture content, the dried chopped stem was then destructed using a commercial blending machine, and the powdered stem was immersed in each solvent with the ratio of 1:3, stored in a dark room, and was shacked every 5 hours for 3 days. After 3 days, the crude extract was filtered using filter paper (Whatman No. 1), concentrated using a rotary evaporator at the desired temperature (depending on the solvent), and the thick extract was stored in the refrigerator for further use.

GC-MS Analysis

GC-MS analysis was conducted using the instrument of Agilent 7890B GC System coupled with a 5977A Mass Selective Detector, equipped with an HP-5MS column (30 m x 0.25 mm, 0.25 µm film thickness). The injector temperature was set to 250°C, helium was used as carrying gas at a flow rate of 1.0 $mLmin^{-1}$ with a split ratio of 10:1. The oven temperature program was set to 50°C (2 min), increased to 150°C at $10°Cmin^{-1}$ (2 min), then to 280°C at $10°Cmin^{-1}$ (10 min). MS conditions included an ion source temperature of 230°C, a quadrupole temperature of 150°C, and a mass range of 50-550 m/z. The identified compounds were compared with the NIST library database for confirmation.

Antimalarial Assay (in vitro)

The in vitro antimalarial activity test utilized the Plasmodium falciparum strain 3D7, known for its sensitivity to chloroquine. Giemsa staining microscopy method was employed in this test and the procedure of evaluation is as follows.

Preparation of Test Samples and Targeted Parasite

A total of 10 mg of the sample was dissolved in 1000 µL of DMSO (stock solution, concentration 10,000 µg/mL). Subsequently, serial dilutions were made from the stock solution to obtain final concentrations of 100, 50, 10, 1, 0.1, and 0.01 µg/mL. The parasites in this test were synchronized (Ring stage) with a parasitemia of approximately 1% (hematocrit 5%).

Testing Procedure

A total of 2 µL of the test solution at various concentrations was added to each well (96-well plate), followed by 198 µL of the parasite (Duplo). The test wells were subsequently placed in a chamber and supplied with a gas mixture (5% O_2, 5% CO_2, and 90% N_2). The chamber containing the test wells was incubated for 48 hours at 37°C. The culture was then harvested, and thin blood smears were prepared and stained with 20% Giemsa.

Data Analysis

The prepared blood smears were assessed through the enumeration of infected erythrocytes per 1000 normal erythrocytes under microscopic examination. Subsequently, this dataset is utilized to ascertain the Percentage Growth and Percentage Inhibition. The Percentage Growth is calculated employing the following formula:

$$\text{Percentage growth} = \text{Percentage Parasitemia} - D_0$$

Where D_0 = Percentage growth at time 0
The percentage of inhibition is calculated using the equation:

$$Inhibition\ (\%) = 100 - \left(\frac{(X_s)}{(X_c)} x100\%\right)$$

Where X_s is the percentage growth in the sample, and X_c is the percentage growth in the negative control

RESULTS AND DISCUSSION

Chemical Compounds Analysis using GC-MS

The chemical compounds contained in the *S.cumini* stem which was extracted using varied solvents were analyzed using GC-MS. The solvents show different abilities in dissolving chemical constituents based on the chemical properties of each solvent, especially its polarity. Polar solvents like ethanol and methanol show tendencies to dissolve polar compounds while nonpolar solvents such as hexane and toluene like to dissolve fat and oil components. However, to dissolve a wider range of compounds, semipolar solvents like ethyl acetate and iso-propanol are usually employed in the extraction process. Four solvents used in this research to extract the active components of the *S. cumini* stem exhibit remarkable differences in the number of solvents as evidenced by the GC-MS chromatograms.

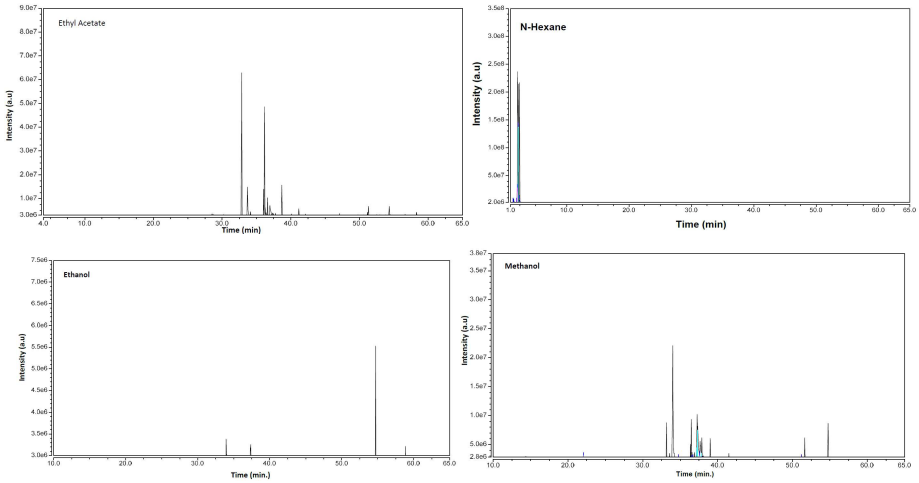

Fig.-1: Chromatograms of *S.cumini* Stem Extracts at Varied Solvents

Figure-1 depicts chromatograms of the *S. cumini* stem extracted using four different solvents. The ethyl acetate and methanol exhibit more peaks indicating their capability of dissolving more compounds than the solvents of ethanol and hexanes. Ethyl acetate and methanol also show quite similar patterns of retention time (RT). While n-hexane and ethanol indicate the opposite pattern, n-hexane shows peaks at lower retention time (1-5 min) which is probably due to the presence of small molecular weight compounds, whereas the ethanol shows peaks at the RT between 30 and 60 min. The GC analysis was coupled with mass spectroscopy (MS) to determine the compounds, and the results indicate that the number of compounds detected in the extract of ethyl acetate, n-hexane, ethanol, and methanol are 23, 7, 5, and 21 respectively. All compound names and retention times are listed in Table-1, and 4 compounds with potential antimalarial activity are depicted in Fig.-3.

Table-1: Chemical Constituents of *S. cumini* Extracts Analyzed using GCMS

Ret. Time (min)	Compound Name and solvents
	Ethyl acetate
28.521	Cyclopropanebutanoic-acid, 2-[[2-[[2-[(2 pentylcyclopropyl) methyl] cyclopropyl] methyl]-cyclopropyl]methyl]-, methyl ester
30.16	3-Trifluoroacetoxypentadecane
32.84	Hexadecanoic acid, methyl ester
33.687	n-Hexadecanoic acid
34.143	3-Trifluoroacetoxypentadecane
36.044	Methyl 9-cis,11-trans-octadecadienoate

36.163	9-Octadecenoic acid (Z)-, methyl ester
36.262	trans-13-Octadecenoic acid, methyl ester
36.394	Phytol
36.612	Methyl stearate
36.949	Oleic Acid
37.775	3-Trifluoroacetoxypentadecane
38.69	Retinal, 9-cis-
41.159	1-(1,3-Dimethyl-buta-1,3-dienyl)-3,7,7-trimethyl-2-oxa bicyclo[3.2.0]hept-3-ene
42.125	Z,Z,Z-4,6,9-Nonadecatriene
47.081	3-Trifluoroacetoxypentadecane
49.764	3-Trifluoroacetoxypentadecane
51.026	9,10-Secocholesta-5,7,10(19)-triene-3,25,26 triol, (3ß,5Z,7E)-
51.165	3-Trifluoroacetoxypentadecane
51.291	Stigmasta-3,5-diene
54.322	ß-Sitosterol
56.628	Cucurbitacin b,25-desacetoxy-
58.281	1-Heptatriacotanol
n-Hexane	
1.416	2-Piperidinone, N-[4-bromo-n-butyl]-
1.518	Methyl Alcohol
1.97	Trichlorodocosyl-sylane
2.048	3-methyl-Pentene
2.109	n-Hexane
2.266	2-Ethyl-oxetane
2.412	Cyclopentane, methyl-
Ethanol	
33.94	n-Hexadecanoic acid
37.365	E-9-Tetradecenoic acid
37.627	Z-8-Methyl-9-tetradecenoic acid
54.701	ß-Sitosterol
58.84	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl) cyclohexane
Methanol	
22.082	Santalol, cis,a-
33.156	Hexadecanoic acid, methyl ester
33.564	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
34.003	n-Hexadecanoic acid
34.73	7-Methyl-Z-tetradecen-1-ol acetate
36.359	11,14-Eicosadienoic acid, methyl ester
36.465	trans-13-Octadecenoic acid, methyl ester
36.57	2-Hexadecenoic acid, methyl ester, (E)-
36.706	2-Hydroxyhexadecyl butanoate
36.921	Heptadecanoic acid, 10-methyl-, methyl ester
37.149	11,14-Eicosadienoic acid, methyl ester
37.247	trans-13-Octadecenoic acid
37.325	cis-Vaccenic acid
37.649	Octadecanoic acid
37.88	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl],methyl este
39.006	2-[5-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-[1,4]benzoquinone
41.495	2-Cyclopenten-1-one, 3-methyl-2-(2,4-pentadienyl)-, (Z)-
49.886	30-Norlupan-28-oic acid, 3-hydroxy-21-methoxy-20-oxo-, methyl ester, (3ß)-
51.185	1-Heptatriacotanol
51.634	Stigmasta-3,5-diene
54.76	Sitosterol

The extraction of chemical compounds from the *S. cumini* stem using different solvents; ethyl acetate, n-hexane, ethanol, and methanol. The finding reveals notable differences in the types of compounds each solvent can dissolve, as shown in the GC-MS analysis data. Ethyl acetate, with its moderate polarity, effectively extracts a diverse range of compounds. Notable examples include Cyclopropanebutanoic acid derivatives with a retention time of 28.521 minutes, Hexadecanoic acid methyl ester at 32.84 minutes, and Phytol at 36.394 minutes. This solvent also dissolves several methyl esters of fatty acids, such as Methyl 9-cis,11-trans-octadecadienoate (36.044 minutes), and compounds like 3-Trifluoroacetoxypentadecane appearing multiple times, indicating its versatility in extracting both polar and non-polar compounds[15] Ethanol shows a similar pattern, effectively extracting compounds such as n-Hexadecanoic acid (33.94 minutes) and ß-Sitosterol (54.701 minutes), highlighting its utility in obtaining a broad spectrum of bioactive molecules. Ethanol's capability to dissolve both polar and non-polar compounds makes it a versatile choice for herbal extractions, as evidenced by its ability to extract E-9-Tetradecenoic acid (37.365 minutes) and Z-8-Methyl-9-tetradecenoic acid (37.627 minutes)[16,17] Methanol, being highly polar[18,19], is particularly effective at extracting polar compounds, as demonstrated by the presence of Hexadecanoic acid methyl ester (33.156 minutes) and Santalol, cis, a- (22.082 minutes) in the GC-MS data. It also extracts various methyl esters of fatty acids efficiently, including 11,14-Eicosadienoic acid methyl ester (36.359 minutes) and Heptadecanoic acid, 10-methyl-, methyl ester (36.921 minutes). The highly polar nature of methanol allows for the extraction of compounds that other less polar solvents might miss[20] On the other hand, n-hexane, a non-polar solvent[21] predominantly extracts non-polar compounds such as n-Hexadecanoic acid (33.94 minutes) and various hydrocarbons like Trichlorodocosyl-silane (1.97 minutes). The presence of short retention time compounds like 2-Piperidinone, N-[4-bromo-n-butyl]- (1.416 minutes) further underscores its role in dissolving smaller, volatile non-polar molecules [22]. This specialization makes n-hexane less diverse in its extraction profile compared to the other solvents but highly effective for specific types of non-polar compounds. These differences highlight the importance of solvent selection based on the target compounds in phytochemical studies and the extraction of bioactive components from *S. cumini* stem.

Antimalarial Assay

Conducting antimalarial tests using plant extracts involves meticulous preparation, extraction, and evaluation processes[4] The importance of this research lies in the urgent need for new antimalarial treatments and the rich potential of plants to provide novel therapeutic compounds. By exploring the naturally occurring chemicals in plants like *S. cumini*, researchers aim to discover effective and sustainable solutions to combat malaria, contributing significantly to global health efforts.

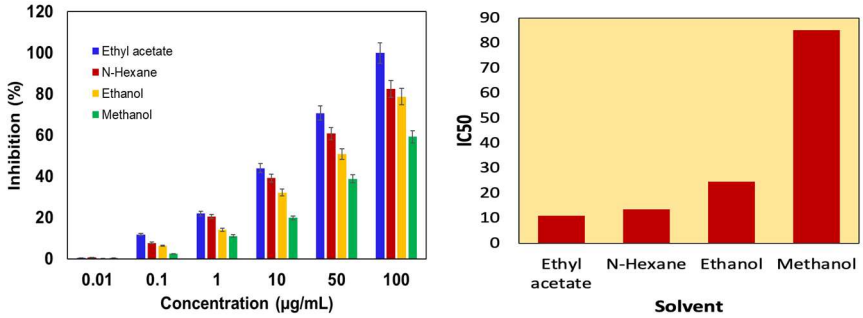

Fig.-2: Inhibition Percentage of Parasitemia by *Syzygium Cumini* Stem Extracted Using Four Different Solvents and their IC_{50} Values

The graph illustrates the inhibition percentage of Plasmodium falciparum growth by *S. cumini* stem extracts at various concentrations (0.01 to 100 µg/mL) using four different solvents: ethyl acetate, n-hexane, ethanol, and methanol. For all solvents, the inhibition percentage increases with the concentration of the extract. The inhibition percentage of all solvents increases with the concentration of the extract. The extract of ethyl acetate shows the highest inhibition at almost all concentrations, particularly at higher concentrations (50 and 100 µg/mL), reaching nearly 100% inhibition at 100 µg/mL. Methanol extract shows the least inhibition among the four solvents, indicating lower effectiveness in extracting antimalarial compounds from *S.*

cumini stems. The high inhibition observed with ethyl acetate suggests that it is the most effective solvent for extracting antimalarial compounds from *S. cumini* stems. This might be due to its ability to dissolve a wider range of bioactive compounds or more effective extraction of specific antimalarial agents[23]. N-hexane also proves to be a strong solvent, indicating its suitability for extracting certain non-polar compounds with antimalarial activity. On the other hand, ethanolic extract and Methanolic extract possessed the relatively lower inhibition observed with methanol suggesting it is less effective in extracting the antimalarial compounds from the stem, possibly due to its polarity or the nature of the compounds it extracts. The findings suggest that ethyl acetate extracts of *S. cumini* stems could be further investigated and potentially developed as a potent antimalarial treatment. The data also highlight the importance of solvent selection in the extraction process for optimizing the yield of bioactive compounds. The graph provides clear evidence of the differential effectiveness of various solvents in extracting antimalarial compounds from *S. cumini* stems. Ethyl acetate emerges as the most effective solvent, followed by n-hexane, ethanol, and methanol. This information could be crucial for future research and development of antimalarial treatments based on *S. cumini* extracts.

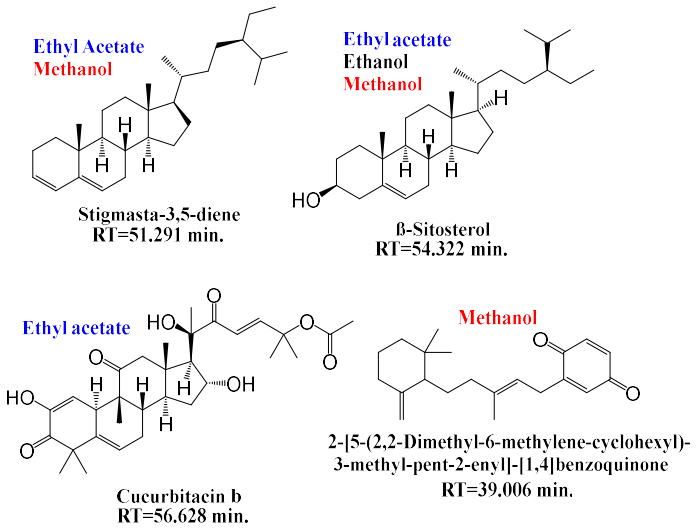

Fig.-3: Compounds with Antimalarial Activity Potential

Figure-3 depicts compounds with a tendency to active constituents with anti-malarial properties due to their chemical properties and structural relationship with chloroquine. Stigmasta-3,5-diene which contained the ethyl acetate and methanol extracts is a sterol, a type of steroid alcohol that is structurally similar to cholesterol and found predominantly in plant cells[24] Sterols play a crucial role in maintaining cellular membrane integrity and fluidity. The disruption of membrane functions can be detrimental to cell survival. While specific studies on the antimalarial properties of stigmasta-3,5-diene are limited, its structural similarity to other biologically active sterols suggests potential antimalarial activity. Sterols can interfere with the Plasmodium parasite's cellular membranes, potentially disrupting the parasite's lifecycle[25] To fully understand its antimalarial potential, further research involving in vitro (test tube) and in vivo (animal model) studies is necessary to evaluate its efficacy and safety against malaria. Another chemical with antimalarial potential use is sitosterol found in all extracts except n-hexane. Sitosterol is plant sterol similar to cholesterol and is abundant in seeds, nuts, and various plants. [26–28] Known for its diverse biological activities[28], including anti-inflammatory and immunomodulatory effects, sitosterol has been studied for various health benefits[27] By enhancing the host's immune response, sitosterol could potentially help in reducing the parasite load in infected individuals[10]. However, to establish sitosterol as a viable antimalarial agent, comprehensive studies focused on its direct and indirect effects on Plasmodium parasites are required. The cucurbitacin B, 25-desacetoxy which is contained in the ethyl acetate extract belongs to the cucurbitacins class of highly oxygenated triterpenoids found in cucurbitaceous plants like cucumbers and melons. Known for their potent cytotoxic properties, cucurbitacins have demonstrated various

biological activities, including anticancer, anti-inflammatory, and antimicrobial effects. Cucurbitacin B, 25-desacetoxy, in particular, has shown promise in preliminary antimalarial studies. [2930] Given its potent biological activities, Cucurbitacin B, 25-desacetoxy could be a valuable addition to the resource of antimalarial agents, potentially in combination with other drugs to enhance efficacy and reduce the risk of resistance development. Comprehensive studies are needed to fully understand its potential and to optimize its use in malaria treatment. The compounds of Stigmasta-3,5-diene, Sitosterol, 2-[5-(2,2-Dimethyl-6-methylene-cyclohexyl)-3-methyl-pent-2-enyl]-[1,4]benzoquinone, and Cucurbitacin B, 25-desacetoxy each exhibit unique structural features and biological activities that suggest potential antimalarial properties.

CONCLUSION

The study demonstrates the significant influence of solvent polarity on the extraction efficiency of bioactive compounds from *S. cumini* stem, as evidenced by the GC-MS analysis and antimalarial assays. Ethyl acetate, a semi-polar solvent, was the most effective in extracting a wide range of compounds, including those with potential antimalarial activity, as indicated by its superior inhibition of Plasmodium falciparum growth across various concentrations. These findings underscore the importance of selecting appropriate solvents to optimize the extraction of specific bioactive compounds from plant materials. Ethyl acetate's performance suggests it is a promising solvent for isolating antimalarial agents from *S. cumini* stem, warranting further investigation into its potential for developing effective and sustainable antimalarial treatments.

ACKNOWLEDGMENTS

The authors acknowledge the Director of Aceh Health Polytechnics and the Tropical Disease Diagnostic Center of Airlangga University, Surabaya for the antimalarial test.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

Munira https://orcid.org/0000-0002-5881-0386
Noni Zakiah https://orcid.org/0009-0000-8999-7115
Muhammad Nasir https://orcid.org/0000-0001-6871-8770

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