Research Article

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Antimalarial Activity of *Andrographis Paniculata* Ness’s N-hexane Extract and Its Major Compounds

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Abstract: *Andrographis paniculata* Ness is one of the plants that is under explored and could contain potentially active substances to serve as an antimalarial. Structure investigation of major compounds that have responsibility for the antimalarial activity of *Andrographis paniculata* Ness’s n-hexane extract is very important so that it is known whether the antimalarial activity is synergistic or from the major compounds. Menthol and dioctyladipate as major component from n-hexane extract of *Andrographis paniculata* Ness have been successfully isolated using simple and inexpensive methods. The solvent system used are n-hexane : ethyl acetate (10:1) and n-hexane: chloroform (10:3) consecutively either for column or preparative thin layer chromatography. FT-IR, $^1$H-NMR and GC-MS were used to determine the structure of compounds. The activity of n-hexane extract, menthol and dioctyladipate related to the inhibition of heme polymerization have been done comprehensively. The inhibition of heme polymerization activity of isolate compounds and n-hexane extract are classified to a good level (dioctyl adipate $IC_{50} = 1.15 \pm 0.41 \text{ mg/mL}$, menthol $IC_{50} = 0.31 \pm 0.01 \text{ mg/mL}$, and n-hexane extract $IC_{50} = 0.07 \pm 0.03 \text{ mg/mL}$) and potentially as antimalarial. From the $IC_{50}$, the antimalarial activity of *Andrographis paniculata* Ness’s n-hexane extract is working synergistically, not by the major compounds.

Keywords: antimalarial, *Andrographis Paniculata* Ness, n-hexane extract, menthol, dioctyladipate.

1 Introduction

Malaria continues to be a significant health problem and deadly parasitic disease across the world. During the last 100 years, the world has not given a clear contribution to the curing the disease [1]. The global commitment to the Millennium Development Goals (MDGs) puts malaria eradication into one of the common goals to be achieved. With the end of the MDGs by 2015, the commitment continues through Sustainable Development Goals (SDGs) whereby the specific goal of SDGs is to end the epidemic of neglected-tropical, tuberculosis and malaria diseases by 2030. The Indonesian Ministry of Health stated that malaria was one of the diseases, which was targeted to reduce the Annual Parasite Incidence (API) from 2 to 1 per 1000. Although nationally in 2011-2015 there was a decrease in the incidence of malaria, there are still areas with a high incidence of malaria especially in eastern Indonesia. Therefore effective efforts to address malaria problem are urgently required [2].

Drug development is important in malaria control due to the critical situation of drug resistance of *P. Falciparum*. Chloroquine, primaquine and pyrimethamine were developed and synthesized in Western countries in the 1940s and used as antimalarials. The resistance of chloroquine to *Plasmodium falciparum* was found and spread in the early 1960s [3]. During the Vietnamese War in the 1960s, there was an increase of incidence of chloroquine resistant. This situation encouraged many researchers to find and develop alternative antimalarials that were more effective to treat malaria than chloroquine.
In the late 1960s, Chinese scientists investigated some active compounds to solve the malaria problem from different medicinal herbs and their chemical structures were also determined and identified. It was a totally new agent: artemisinin. Therefore, the drug is not used alone as antimalarial but in combination with another antimalarial drug. The poor solubility and low curative rate of artemisinin stimulated the researchers to synthesize artemisinin derivatives (artemether, artesunate, and dihydroartesinin). Beside the isolation method, chemical synthesis was an important method for developing new antimalarials. The synthetic antimalarials (piperazine, pyronaridine, lumefantrine and naphthoquine) were developed as new antimalarials between the 1960s and the 1980s [4].

The occurrence of parasitic resistance to malaria drugs (chloroquine and artemisinin) and the spread of resistance then encouraged researchers to find new antimalarial drugs which are more efficient and effective [5]. It is necessary to find new antimalarials, especially from medicinal plants. *Andrographis paniculata* Ness is one of the plants that is under explored as a useful herbal medicine. Recent research suggests that the plant contains potential active substances to serve as repellent, immunomodulators, hepatoprotectors, antibacterials, anti-inflammatory, anticancer, and antimalarials [6], [7], [8]. Andrographolide is the main compound of *Andrographis paniculata* and is active as an antimalarial [9]. However, it is necessary to search further for other potential compounds of *Andrographis paniculata* which also have potential as antimalarials.

The development of *Andrographis paniculata*’s extract as antimalarials have been done very well, not only on in vitro wherein using both *Plasmodium* culture but also in vivo using experimental animals that have parasitic infection [10], [11]. Study of heme polymerization inhibition activity assay using *Andrographis paniculata*’s extract has been conducted [12]. Heme polymerization inhibition activity assay is one method to determine the mechanism of action of a compound as an antimalarial. The mechanism that occurs is the interaction between the test material with the heme electrolyte system and or active groups in the test material which binds to the iron ion on heme [13]. Chloroquine as an antimalarial drug is also through inhibition of heme polymerization [14].

All of the *Andrographis paniculata* Ness’ extracts with different polarity levels have the capability to inhibit heme polymerization. The IC₅₀ values are 2.19 ± 0.09 mg/mL for n-hexane extract (nonpolar), 1.24 ± 0.01 mg/mL for ethyl acetate extract (semipolar) and 1.16 ± 0.02 mg/mL for ethanol extract (polar). All extracts are classified as active [12]. This provides an opportunity for the isolation process to find the main compound which is responsible for the activity of the extracts. If we can find the main compound responsible for the antimalarial activity of the n-hexane extract, then in the future a synthesis approach can be made to save raw materials and costs. It is very important to know whether the antimalarial activity of this extract is synergistic or originates from its main compound. Therefore, this study conducted some isolation of major compounds from *Andrographis paniculata* Ness’s n-hexane extract (origin from Yogyakarta) and continued with heme polymerization inhibition activity assay from the n-hexane extract and isolate.

## 2 Experimental Detail

### 2.1 Materials

All chemicals used were of analytical grade from JT Baker (n-hexane, ethyl acetate, ethanol, and chloroform), Merck and Co. Inc (methanol, acetic acid, acetonitrile, DMSO, silica gel 60 (0,063 – 0,200 mm), silica gel 60 PF₂₅₄ containing gypsum, Na₂SO₄, H₂SO₄, NaOH, and hematin porcine). The substrate with technical grade is *Andrographis paniculata* Ness and chloroquin in drug (Mexaquin Chloroquine).

The instrument used in this study are laboratory glassware, column, FTIR Thermo Nicolet Avatar 360, Agilent 500 MHz NMR spectrometer, GC-MS QP2010S (Shimadzu), Elisa reader (Thermo Scientific), Incubator (Memmert), Centrifuge (Hettich Rotina 22R), Analitical measure (Fujitsu), Rotary evaporator (Heidelberg Laborata 4000 efficient), Shaker (SCILOGEX SK-O330-Pro), chamber, UV lamp, micropipette (Eppendorf), microtube 1.5 mL, and 96 well microplate.

### 2.2 Procedure

*Andrographis paniculata* Ness’s n-hexane extract prepared from *Andrographis paniculata* Ness’s plant by maceration method using n-hexane as solvent. The n-hexane extract was separated by using column chromatography with the best solvent system i.e. n-hexane : ethyl acetate (10:1) to produce fraction A and B with great separation (Figure 1).

Afterward, fraction A was separated by using column chromatography (n-hexane : ethyl acetate (10:1) and n-hexane: chloroform (10:3) and preparative thin layer chromatography(n-hexane:chloroform(10:3)respectively).
to get dioctyladipate. Fraction B was separated by using column chromatography (n-hexane : ethyl acetate (10:1), n-hexane: chloroform (10:3), n-hexane: diethyl ether (10:2) and preparative thin layer chromatography (n-hexane: chloroform (10:3) respectively) to get menthol.

All compounds were observed by using UV-lamp with 254 and 366 nm of wavelength. In the n-hexane extract and isolated compounds, phytochemical screening was performed i.e. alkaloids, flavonoids, terpenoids, and steroids screening. Isolated compounds were checked by using FT-IR, $^1$H-NMR and GC-MS for determination of the compound. The conditions in the GC-MS analysis are column oven temperature 70°C, injection temperature 300°C with helium as carrier gas, and EI method on MS analysis. Analytical TLC was carried out using pre-coated silica gel plates (Merck TLC silica gel 60 F254). IR spectra were recorded on a Thermo Nicolet Avatar 360 using a NaCl cell; $\tilde{\nu}$ in cm$^{-1}$. $^1$H-NMR spectra were recorded using a Agilent 500 MHz NMR spectrometer. Chemical shifts are reported in ppm relative to CHCl$_3$ ($\delta$(H) 7.26) in CDCl$_3$ for $^1$H-NMR. Splitting patterns are designated as s, d, t, q, and m, indicating singlet, doublet, triplet, quartet, and multiplet, respectively.

Heme polymerization inhibition activity assay were performed by In Vitro method using [13] a method which modified at levels of hematin solution and the level of test sample. Series of test sample levels were made in 10% DMSO with a concentration of 5; 2.5; 1.25; 0.625; and 0.3125 mg/ mL. Each level was made as much as 300 µL. The testing process was carried out by placing 100 µL of 1 mM hematin solution in 0.1 M NaOH into Eppendorf tubes, then adding 50 µL of test material with various levels, namely 5; 2.5; 1.25; 0.625; and 0.3125 mg/ mL. Replication is done 3 times for each level. To start the heme polymerization reaction, 50 µL of glacial acetic acid solution (pH 2.6) was added to the Eppendorf tube which contained hematin solution and samples, then incubated at 37°C for 24 hours. Positive control used was Mexaquin antimalarial drug, while the negative control was 10% DMSO. After the incubation ended, the Eppendorf tube was centrifuged at 8000 rpm for 10 minutes. The supernatant was removed and the precipitate was washed 3 times with 200 µL of DMSO 100%. At each wash, centrifugation speed of 8000 rpm is carried out for 10 minutes. The obtained sediment was added 200 µL of 0.1 M NaOH. Every 100 µL of the solution obtained was put into a 96 microplate well and read the absorbance using Elisa Reader at a wavelength of 405 nm. Heme polymerization inhibitory activity is expressed by IC$_{50}$ values calculated by probit analysis using SPSS version 23. This assay was performed to n-hexane extract, isolated compounds and chloroquin in drug (Mexaquin Chloroquine).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and Discussion

This study was classified in explorative research. The results gave information about major compounds from *Andrographis paniculata* Ness's n-hexane extract and their antimalarial activity. In the first exploration, the class of compound was investigated by phytochemical screening. N-hexane extract collected in dark green oil with 0.4% of yield. Furthermore, there are two major compound which can be collected by chromatography process using n-hexane : ethyl acetate (10:1) and n-hexane: chloroform (10:3) as eluent. The first major compound is more nonpolar than another one with 7% of yield from n-hexane extract and isolated from *Fraction A*, called dioctyladipate.

The second major compound is more polar with 0.35% of yield from n-hexane extract and isolated from *Fraction B*, called menthol. The phytochemical screening also performed for isolate compounds. The results of phytochemical screening i.e. alkaloids, flavonoids, terpenoids, and steroids screening for all compounds are shown in Table 1.
Antimalarial Activity of *Andrographis Paniculata* Ness’s N-hexane Extract and Its Major Compounds

There are 3 groups of compounds in n-hexane extract. This phenomenon is very unusual, but occurs in the extraction process where the alkaloid and flavonoid compounds can be extracted in n-hexane. Therefore, it can be predicted that in the process of separation and purification will continue to take a long time because it still has to separate the main compounds from 3 groups of compounds, alkaloids, flavonoids and terpenoids. This condition also makes the yield of the main compounds (first and second major compound) in the n-hexane extract very small.

In the second exploration, FT-IR, \(^1\)H-NMR and GC-MS analysis were performed on both major compounds to determine the purity and structure of the compounds. Structure elucidation of first major compound, dioctyladipate was performed by means of FT-IR (Figure 2), \(^1\)H-NMR (Figure 3), GC (Figure 4a) and MS (Figure 4b). For dioctyladipate, FT-IR (neat) : 2923, 2852, 1710, 1262 and 1033 cm\(^{-1}\) indicating the presence of C\(_{sp3}\)-H, C=O and asymmetric and symmetric C-O-C.

First major compound, dioctyladipate was analyzed using \(^1\)H-NMR and gives spectrum results (Figure 3): \(^1\)H-NMR (CDCl\(_3\), 500 MHz): 0.88 (6H, t), 1.29 (12H, m), 1.31 (4H, m), 1.43 (4H, m), 1.62 (4H, m), 1.64 (4H, m), 2.32 (4H, t), and 5.34 (4H, t). The spectrum displayed in Figure 3. By the \(^1\)H-NMR spectrum results, it could be stated that dioctyladipate have been successfully isolated as yellow oil. However, there are still many impurities.

The molecular formula was determined to be C\(_{22}\)H\(_{42}\)O\(_4\) based on \(^1\)H-NMR and GC-MS data (M\(^+\), calculated for being 370). The results of GC-MS analysis are shown in Table2 for dioctyladipate as first major compound. The chromatogram and mass spectra also presented in Figure 4.

From the result analysis of first the major compound, there are still many impurities (9 impurity compounds). However, the results concluded that the first major compound is dioctyladipate. This compound was classified as glyceride compound. When associated GC-MS analysis (Table 2 and Figure 4) with the results of phytochemical analysis (Table 1), there appears to be a mismatch between the results of phytochemical analysis with GC-MS analysis. This is because the main

### Table 1: Phytochemical screening of n-hexane extract and major compounds.

| Sample    | Phytochemical screening | Result |
|-----------|-------------------------|--------|
| N-hexane extract | alkaloids | +       |
|            | flavonoids | +       |
|            | terpenoids | +       |
|            | steroids  | -       |
| Fraction A | alkaloids  | -       |
|            | flavonoids | -       |
|            | terpenoids | +       |
|            | steroids  | -       |
| Fraction B | alkaloids  | -       |
|            | flavonoids | -       |
|            | terpenoids | +       |
|            | steroids  | -       |

![Figure 2: FT-IR spectrum of the dioctyladipate (red) and menthol (black).](image)

![Figure 3: \(^1\)H-NMR spectrum of dioctyladipate.](image)
Table 2: Result of analysis of dioctyladipate based on similarity index on GC-MS.

| Peak | Retention time (min) | %area | M*  | Name of compound                        | Class of compound |
|------|----------------------|-------|-----|-----------------------------------------|-------------------|
| 1    | 16.672               | 3.35  | 128 | 2-(2-butoxyethoxy) ethanol              | Alcohol           |
| 2    | 32.041               | 4.67  | 218 | 2-methyl-5-(1,2,2-trimethylcycloamyl)   | Terpenoid         |
| 3    | 36.237               | 1.61  | 277 | Phytol                                  | Terpenoid         |
| 4    | 42.881               | 2.40  | 222 | Oxirane undecanoic acid                 | Carboxylic acid   |
| 5    | 44.717               | 67.35 | 370 | Dioctyladipate                          | Glyceryde         |
| 6    | 48.417               | 3.26  | 383 | Cholesta-8,24-dien-3-ol                 | Triterpenoid      |
| 7    | 48.774               | 2.14  | 383 | 1,1'-1-(2,2-dimethylbutyl)-1,3-propanediyl bis-cyclohexane, | Alkane            |
| 8    | 52.098               | 6.12  | 253 | 4,5-diethyl-2,3-dihydro-2,3-dimethyl furan | Alkene            |
| 9    | 52.634               | 6.71  | 282 | 17-Pentatriacontene                     | Alkene            |
| 10   | 54.784               | 2.38  | 355 | Neryl linalool                          | Monoterpenoid     |

Figure 4: Chromatogram (a) and mass spectra (b) from first major compound, dioctyladipate.
compound, dioctyl adipate, is not including terpenoid group. However, when examined more deeply, the actual positive results of terpenoid on phytochemical analysis obtained from the content of impure compounds that still exist. From GC-MS analysis, it is clear that there are still 4 impurity compounds classified as terpenoids. From the MS analysis (Figure 4b), the spectra have 96% similarity index (SI) and it can be directly determined the name of the compound, as well as its structure.

In the third exploration, structure elucidation of second major compound, menthol was performed by means of FT-IR (Figure 2), 1H-NMR (Figure 5), GC (Figure 6a) and MS (Figure 6b). For menthol, FT-IR (neat): 3242, 2923, 2869, 1044 cm⁻¹ indicating the presence of –OH, Cₛ秵-H, and C-O alcohol. The 1H-NMR gives spectrum results (Figure 5): 1H-NMR (CDCl₃, 500 MHz): 0.78-0.80 (3H, d), 0.89-0.92(6H, dd, J= 6, 8 Hz), 0.94-1.02 (2H, m), 1.06-1.12 (2H, m), 1.37-1.42 (1H, m), 1.46 (1H, s), 1.57-1.66 (2H, m), 1.93-1.96 (1H, m), 2.112.20 (1H, m), and 3.36-3.41(1H, qd, J= 4, 4.2 Hz). The spectrum displayed in Figure 5. By the 1H-NMR spectrum results, it could be stated that menthol have been successfully isolated as white crystals. The molecular formula was determined to be C₆₀H₁₀O based on 1H-NMR and GC-MS data (M⁺, calculated for being 156). The results of GC-MS analysis are shown in Table 3 for menthol as second major compound. The chromatogram and mass spectra also presented in Figure 6.

In contrast to the first major compound, GC-MS analysis of the second major compound (Table 3 and Figure 6a) consisted of only 1 peak with 99% of purity. The results concluded that the second major compound is menthol and that compound was classified as terpenoid. This is in accordance with the results of phytochemical analysis (Table 1) where in the analysis obtained that the compound is positive as terpenoid compounds. From the MS analysis (Figure 6b), the spectra have 90% similarity index (SI) and it can be directly determined the name of the compound, as well as its structure.

The M⁺ from MS analysis of dioctyladipate and menthol were missing. This phenomena can be explain

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**Table 3:** Result of analysis of second major compound based on similarity index on GC-MS.

| Peak | Retention time (min) | %area | M⁺ | Name of compound | Class of compound |
|------|----------------------|-------|----|------------------|-------------------|
| 1    | 39.588               | 99    | 156| Menthol          | Monoterpenoid     |
by fragmentation rules. Intensity of $M^+$ will decrease or invisible for branched and high molecular weight compound. Beside that, when ionization process, the molecules will be fragmented in the branches of their structure and will form more stable ions and these ions are seen in MS spectra. This is the reason why $M^+$ from dioctyladipate (370) and menthol (156) are invisible.

After successfully in determining the structure, the exploration continued to HPIA assay. Hematin will polymerize into β-hematin crystals in acidic condition. Based on the antimalarial mechanism, the candidate will reduce β-hematin crystals formation. The IC$_{50}$ values of the isolate compound, negative and positive controls were written in Table 4. The results showed respectively that n-hexane extract, dioctyl adipate, menthol and positive control chloroquin in drug (Mexaquin Chloroquine) have IC$_{50}$ values 0.07 ± 0.03 mg/mL, 1.15 ± 0.41 mg/mL, 0.31 ± 0.01 mg/mL and 0.35 ± 0.13 mg/mL.

According to [13], a compound could be considered to have heme polymerization inhibitory activity if having IC$_{50}$ values were lower than 12 mg/mL. However, the IC$_{50}$ of dioctyl adipate was higher than positive control chloroquin in drug (Mexaquin Chloroquine) but still lower than 12 mg/mL. This is allegedly because dioctyl adipate does not have hydroxyl groups that can interact with the heme’s iron ion, while chloroquine and menthol have the group.

If the IC$_{50}$ value of n-hexane extract from Andrographis paniculata Ness, and the major compounds contained i.e. menthol and dioctyladipate compared to another antimalarials compound that have been found before, it can be concluded that menthol and dioctyladipate is quite good as antimalarials although not better than andrographolide which is the main compound of Andrographis paniculata Ness. However, n-hexane extract from Andrographis paniculata Ness has a better value and very potential to be used as an antimalarial wherein the preparation process is quite easy. The results of the toxicity test showed that n-hexane extracts have LC$_{50}$ values is 1,155 μg/mL [12]. Therefore all of the Andrographis paniculata Ness’s extract is safe to use. Previous studies also reported that extracts of Andrographis paniculata

**Figure 6:** Chromatogram (a) and mass spectra (b) from second major compound, menthol.
**Table 4:** The IC$_{50}$ values of n-hexane extract, dioctyl adipate, menthol, negative and positive controls based on HPIA assay.

| Samples                        | Concentration (mg/mL) | Average dose of hemoglobin (mM) | Average percent of inhibition | IC$_{50}$ (mg/mL) |
|--------------------------------|-----------------------|---------------------------------|------------------------------|------------------|
| n-hexane extract of Andrographis paniculata Ness | 5                     | 14.00 ± 3.50                    | 80.520 ± 4.477               |                  |
|                                | 2.5                   | 12.35 ± 2.58                    | 79.635 ± 4.436               |                  |
| Andrographis paniculata Ness   | 1.25                  | 26.46 ± 5.18                    | 67.362 ± 6.246               | 0.07             |
|                                | 0.63                  | 51.78 ± 18.04                   | 53.588 ± 15.571              |                  |
|                                | 0.31                  | 35.75 ± 7.69                    | 58.345 ± 6.44                |                  |
| Dioctyl adipate                | 1.25                  | 193.71 ± 11.99                  | -85.70 ± 11.50               | 1.15             |
|                                | 0.63                  | 122.33 ± 13.92                  | -17.27 ± 13.35               |                  |
|                                | 0.31                  | 95.02 ± 9.70                    | 8.91 ± 9.30                  |                  |
| Menthol                        | 5                     | 15.99 ± 6.86                    | 84.67 ± 6.58                 |                  |
|                                | 2.5                   | 44.94 ± 24.94                   | 56.89 ± 23.92                |                  |
| Mexaquin chloroquine          | 1.25                  | 63.69 ± 16.89                   | 38.91 ± 16.21                | 0.35             |
|                                | 0.63                  | 48.85 ± 8.97                    | 53.15 ± 8.60                 |                  |
|                                | 0.31                  | 87.34 ± 62.26                   | 16.23 ± 59.71                |                  |
| Chloroquine sulfate           | 5                     | 3.29 ± 0.42                     | 79.13 ± 2.70                 |                  |
|                                | 2.5                   | 8.97 ± 4.76                     | 43.09 ± 30.23                |                  |
| chloroquine                    | 1.25                  | 3.35 ± 1.86                     | 78.77 ± 11.80                | 0.35             |
|                                | 0.63                  | 13.32 ± 2.60                    | 15.48 ± 16.44                |                  |
|                                | 0.31                  | 12.32 ± 0.42                    | 21.83 ± 2.64                 |                  |

**Table 5:** Comparison of IC$_{50}$ values of menthol and dioctyl adipate with previous antimalarials.

| Compound                              | IC$_{50}$ (mg/mL) | Plant / Source            | Reference |
|---------------------------------------|-------------------|---------------------------|-----------|
| Chloroquine sulfate                   | 12                | Synthetic                 | [13]      |
| Dioctyl adipate                       | 1.15              | Andrographis paniculata   |           |
| 2,3,4-Trihydroxy-5-methylxanthone     | 0.75              | Synthetic                 | [17]      |
| Mexaquin Chloroquine                  | 0.35              | Drug / synthetic          |           |
| Menthol                               | 0.31              | Andrographis paniculata   |           |
| Andrographolide                       | 0.13              | Andrographis paniculata   | [9]       |
| n-hexane extract of Andrographis paniculata Ness | 0.07          | Andrographis paniculata   |           |
Ness had no toxic effect on Artemia salina larvae [15]. In vivo testing to determine acute toxicity using animal testing of mice also showed that Andrographis paniculata Ness extract included in the safe category with an LD$_{50}$ value more than 5,000 mg/kg BW [16]. Thus all major compounds from n-hexane extracts in this study are safe to use in humans.

The activity of n-hexane extract of Andrographis paniculata is higher than their major compounds or main compounds because there are several compounds in the n-hexane extract wherein having synergistic activity. There are 3 class of compounds included in n-hexane extract (alkaloids, flavonoids and terpenoids). This condition has increased the activity of n-hexane extract to inhibit heme polymerization. The activity can be seen from IC$_{50}$ values of n-hexane extract, which is lower than both major compounds.

The presence of heme polymerization inhibition activity by n-hexane extract and menthol are suspected due to the interaction between hydroxyl groups contained in extracts or isolates with heme iron ions. This is based on research conducted by [13]. Terpenoid group compounds, phenols, and steroids have an inhibitory activity of heme polymerization due to the interaction between compounds with heme’s electronic systems or hydroxyl group bonds with heme iron ions. Although there is no hydroxyl group on dioctyl adipate, this compound still has inhibition activity. This is possible because dioctyl adipate has an ester group in which the oxygen atoms of the ester group can also interact with iron even though the interaction is not as good as the hydroxyl group. The interaction of β-hematin with menthol and dioctyl adipate was described in Figure 5a and 5b.

4 Conclusion

In conclusion, we have presented the isolation of the major component from Andrographis paniculata Ness’s n-hexane extract by using liquid chromatography method. Major compounds that can be isolated i.e. dioctyl adipate and menthol although there are still many impurities on the dioctyl adipate. The inhibition of heme polymerization activity of both compounds are very good (dioctyl adipate IC$_{50}$ = 1.15 ± 0.41 mg/mL, menthol IC$_{50}$ = 0.31 ± 0.01 mg/mL, n-hexane extract IC$_{50}$ = 0.07 ± 0.03 mg/mL while n-hexane extract is more active than both of major compound. The antimalarial activity of Andrographis paniculata Ness’s n-hexane extract is working synergistic, not by the major compounds. In case, menthol and n-hexane extract displayed better antimalarial activity than chloroquine (IC$_{50}$ = 0.35 ± 0.13 mg/mL).

Conflict of interest: Authors declare no conflict of interest.

References

[1] Riley E.M., Wagner G.E., Ofori M.F., Wheeler J.G., Akanmori B.D., Tetteh K., McGuinness D., Bennett S., Nkrumah F.K., Anders R. F., and Koram K. A., Lack of association between maternal antibody and protection of African infants from malaria infection, Infect. Immun., 2000, 68(10), 5856-5863.
Antimalarial Activity of *Andrographis Paniculata* Ness’s N-hexane Extract and Its Major Compounds

[2] Ministry of Health, Malaria, Infodatin Kementerian Kesehatan, Jakarta, 2016, 1-8.

[3] Moore D.V. and Lanier J.E., Observations on studies on two Plasmodium falciparum infections with an abnormal response to chloroquine. Am. J. Trop. Med. Hyg., 1961, 10, 5–9.

[4] Li Y., Yu P. L., Chen Y.X., Li L.Q., Gai Y.Z., Wang D.S., and Zheng Y.P., Synthesis of some derivatives of artemisinine, Chin. Sci. Bull., 1979, 24, 667–669.

[5] Huy N.T., Maeda A., Uyen D.T., Trang D.T.X., Sasai M., Shiono T., Oida T., Harada S., and Kamei K., Simple colorimetric inhibition assay of heme crystallization for high-throughput screening of antimalarial compounds. Antimicrob. Agents Chemother., 2007, 51, 350-353.

[6] Sopi I.I.P. B. and Tallan M.M., Some review medicinal plants used in traditional malaria treatment. SPIRAKEL, 2015, 7(2), 28-37.

[7] Prakoso N.I., Azizah U., Zakiyah Z.N., Nita M.T., Liyanita A., and Suputa, An Investigation of Insect Ovipositing Repellent Activity of *Andrographis paniculata* Ness, *Acacia auriculiformis* and *Piper betle* Linn Leaves Extracts to *Batrocea carambolae*, Eksakta J. Ilmu-ilmu MIPA, Yogyakarta, 2016, 16, 45-54.

[8] Joselin J. and Jeeva S., *Andrographis paniculata*: A Review of its Traditional Uses, Phytochemistry and Pharmacology, Med. Aromat. Plants, 2014, 3 169, 121-131.

[9] Zein U., Fitri L. E., and Sarasih A., Comparative study of antimalarial effect of *Andrographis paniculata* (*Andrographis paniculata* Nees.) as an Antimalarial Drug Study on Oxidative Stress of *Plasmodium berghei* ANKA. Disertasi, Program Doktoral, Universitas Indonesia, Depok. 2014. www.lontar.ui.ac.id.

[10] Widyawaruyanti A., Asrory M., Ekasari W., Setiawan D., Radjaram A., Tumewu L., and Hafid A.F., In vivo antimalarial activity of *Andrographis paniculata* tablets. Procedia Chem., 2014, 13, 101-104.

[11] Basilico N., Pagani E., Monti D., Ollario P., and Taramelli D., A microtitre-based method for measuring the haem polymerization inhibitory activity (HPIA) of antimalarial drugs, J. Antimic. Chem., 1998, 42, 55-60.

[12] Baelmans R., Deharo E., Muñoz V., Sauvain M., and Ginsburg H., Experimental Conditions for Testing the Inhibitory Activity of Chloroquine on the Formation of β-Hematin, Exp. Parasitol., 2000, 42, 55-60.

[13] Mammatha A., Brine shrimp lethality test of *Andrographis paniculata*, Res. J. Pharm. Technol. 2014, 7(7), 743-745.

[14] Katrin E., Susanto, Winarno H., Dry bitter (*Andrographis paniculata* nees) safety which is irradiated by gamma based on its acute toxicity aspects to Swiss Webster mice, Jurnal Sains dan Teknologi Nuklir Indonesia, 2014, 15(2), 103-118.

[15] Fitriastuti D., Jumina and Priatmoko, Heme polymerization inhibition activity (HPIA) assay of synthesized xanthone derivative as antimalarial compound, AIP Conference Proceedings, 2017, 1823, 020120.