# In Vitro Effect of 96% Ethanol Extract of Bitter Herbs (*Andrographis paniculata Nees*) on Heme Detoxification Process of *Plasmodium falciparum* Parasites

Safarianti Safarianti[1], Aty Widyawaruyanti[2], Hilkatul Ilmi[2], Achmad Fuad[2], Indah Tantular[2], Maryatun[1]

[1]*Faculty of Medicine, University of Syiah Kuala, Darussalam, Banda Aceh, Indonesia*

[2]*Institute of Tropical Disease, University of Airlangga, Surabaya, Indonesia*

Keywords: The 96% Ethanol Extract Of Bitter Herbs, *Andrographis Paniculata Nees*, Heme Detoxification Process.

Abstract: Malaria still becomes a global health problem especially in tropical countries and developing countries. The number of malaria infections in Indonesia is still high and it becomes endemic disease in some areas. The high rate incidence of malaria can be caused by the emergence of malaria parasite strains that are resistant to anti malarial drugs. Various efforts have been made to reduce the incidence of malaria, one of which was to develop a new medicinal compounds from natural ingredients such as 96% ethanol extract of bitter herbs (*Andrographis paniculata* Nees). This herb has been proven to have antimalarial activity both in-vitro and in-vivo from previous studies. This study aims to determine the effect of 96% ethanol extract of bitter herbs on heme detoxification process of Plasmodum falciparum through the inhibition of β-hematin formation (synthetic heme). This research is a laboratory experimental research that was conducted in Malaria Laboratory of Institute of Tropical Disease (ITD) University of Airlangga Surabaya. The results showed that 96% ethanol extract of bitter herbs inhibits the formation of β-hematin equal to 61,07 ± 4,69%. It can be concluded that 96% ethanol extract of bitter herbs *(Andrographis paniculate Nees)* are able to inhibit heme detoxification process of *Plasmodium falciparum* parasites.



## 1 INTRODUCTION

96% ethanol extract is a natural herb obtained from bitter plants (Andrographis paniculataNees / *Sambiloto*). This traditional medicinal plant that has long been used empirically as an anti-malarial drug (Widyawaruyanti, 2009). The compound substances are contained with *Sambiloto* herbal plants include diterpenoid lactone, flavonoid, xanton and Andrographolide which are the main active substances of this plant. The level of Andrographolide in the form of ethanol extract 96% is ± 14,91% (Okhuarobo et all, 2014; Safitri EY, 2007).

96% ethanol extract of *Sambiloto* herb are obtained from extraction process of *Sambiloto* powder through maceration process with its solution is 96% ethanol to enable it to draw a range of compounds contained in *Sambiloto* plant (Widyawaruyanti, 2009). Earlier research has indicated that 96% ethanol extracts of *Sambiloto* herbs are active as antimalarial drug against *Plasmodium falciparum* in vitro, and are also active Against *Plasmodium berghei* in-vivo (Widyawaruyanti et al, 2014; Rahman et al, 1999). Other studies show that 96% ethanol extracts of Sambiloto herbs were found to have antimalarial activity at medium-to-moderate categories with inhibitor concentration value (IC) 2,287 μg/ml that inhibits the development of schizont stage (Schizontocide) (Resi, 2013; Widyawaruyanti et al, 2015).

One of the important work targets of antimalarial drugs in killing malaria parasites is by inhibiting the process of heme detoxification from developing into hemozoin crystals. This is a vital process for the parasite and has been validated as one of the antimalarial work targets. Therefore it is necessary to study the effect of 96% ethanol extract of Sambiloto herbs in inhibiting the process of heme detoxification of Plasmodium parasite to figure out



In Vitro Effect of 96 .
DOI: 10.5220/0008791100720075
In *Proceedings of the 2nd Syiah Kuala International Conference on Medicine and Health Sciences (SKIC-MHS 2018)*, pages 72-75
ISBN: 978-989-758-438-1

the mechanism of 96% ethanol extract of Sambiloto herbs in killing parasites.

## 2 RESEARCH METHODOLOGY

This is a laboratory experimental research. The antimalarial herb 96% ethanol extract *Sambiloto* herbs at dose 100 µg / ml is incubated with hemin chloride and then its effect is observed on heme detoxification process by using spectrophotometer. The sample used in this study was hemin chloride which is a synthetic pigment obtained from Sigma®.

The inhibition of the *P. faciparum* heme parasite detoxification process is carried out by synthetically testing the inhibition of β-hematin formation. In this test, the active antimalarial compound of 96% ethanol extract of Sambiloto herbs with the 100 µg / ml concentration obtained from the ITD Science and Technology Research Partnership for Sustainable Development (SATREPS) Laboratory was incubated in acetate buffer with α-chlorohemin (Sigma) solution for 24 hours, with trial of duplication.

### 2.1 Preparing the Test Substance of 96% Ethanol Extract of Sambiloto Herbs

The 96% ethanol extract of Sambilato herbs as the test substance in this study was firstly prepared through the following steps:

- The extract is to be weighed 10 mg of test substance, dissolved in 100 µl DMSO to obtain solution concentration of 100.000 µg / ml as stock solution
- Measure as much as 10 µl of stock solution with pipette then add a complete medium until the volume reaches 500 µl which results in the solution concentration of 2000 µg / ml (Solution A)
- Measure as much as 10 µl of solution A with pipette then add a complete medium until the volume reaches 500 µl which results in the solution concentration of 100 µg / ml (Solution B)

### 2.2 Procedure of synthetic heme detoxification inhibion Test (*ß-Hematin Inhibition Assay*) (Tekwani BL and Walker LA, 2005)

The inhibition test of heme detoxification process is undertaken through the following steps:

- Turn solid natrium acetate into 8M natrium in warm water. Do the mixing continously at 370C in waterbath
- Add 8 M acetic acid until it reaches pH 5.0 value. The addition of acetic acid is to be done at 370C, then incorporate a total of 100 µl of the solution in the falcon tube.
- Add the 96% ethanol extract test substance of 50 µl with 100 µg / ml concentration in DMSO in a falcon tube containing the acetate buffer solution.
- Add a total of 50 µl of hemin chloride 8mM (Sigma) in DMSO to the falcon containing the test substance and acetate buffer solution.
- Undertake incubation for 24 hours at $35^0$C with two replications.
- Centrifugalize β-hematin and insoluble hematin (in the from of pellet) at 3000 rpm for 20 minutes at a temperature of 22 $^0$C, and discard the supernatant.
- Add 200 µl DMSO to the obtained pellet to dissolve unreacted hematin, then re-centrifugalize it and discard the supernatant.
- Add 200 µl NaOH 0.2M to the pellet to convert ß-hematin into a soluble hematin alkaline, then make each sample of alkaline hematin in dilution series with Aqua bidest.
- Measure absorbance using a spectrophotometer at a wavelength of 414 nm.
- Calculate the effect of the test substance on the production of ß-hematin and compare it with the negative control. The negative control used in this study was 50 µl of DMSO solution.

In this study, the data analysis was undertaken descriptively by comparing the group that has been given 96% ethanol extract of sambiloto herbs with the negative control group.The sentence must end with a period.

## 3 RESULTS AND DISCUSSION

The Plasmodium parasite will consume hemoglobin from erythrocyte cells as one of its nutrients for its survival. The stage at which hemoglobin is mostly consumed is the tropozoit stage. Being in erythrocytes, the parasite endocytoses a number of nutrients that are mostly hemoglobin coming from erythrocyte cytosol.

Endocytosis occurs through the process of invagination of parasitic plasma membranes known as sitostoma. Once the endocytosis process is complete, the neck of sitostoma is removed to release the double membrane of the endocytic vesicle that contains the cytoplasm of red blood cells.

The digestion process of erythrocyte cytoplasm is initiated by proteolytic gastrointestinal enzymes in double membranes vesicular transport. The double membrane vesicular transport is thought to originate from the invagination of the parasitic plasma membrane as the outer layer and the parasitophoruos vacuoles membrane as the inner layer.

The result of host cytoplasm endocytosis (80% hemoglobin) will be transported by vesicle transport into the food vacuole and degraded proteolitically into heme and globin in the food vacuole namely an oxygen-rich lysosome-like organelle at the pH between 5-5,5 (Hong-Chang Z et al, 2009; Hoppe HC, 2004).

The Plasmodium parasite consumes only globin as its nutritional source, while the heme is released. Free heme is toxic to the parasite and has the ability to lyse the parasitic membrane of Plasmodium. Therefore the parasite must detoxify the heme through certain mechanisms, one of which is by converting free heme into insoluble crystal hemozoin (Egan TJ, 2007).

The effects of Plasmodium parasite's heme inhibition can be known through β-Hematin-formation inhibition test (ß-Hematin Inhibition Assay). β-hematin has a structure and spectrum identical to hemozoin, in which case the β-hematin formation inhibition test may be used to determine the effect of antimalarial drugs on the detoxification process of heme parasite *P. falciparum*.

ß-hematin can be formed in vitro in acidic atmosphere (acetate buffer solution pH 5.0) of hematin or hemin chloride. Hemin chloride will react to acetate buffer and form an insoluble β-hematin after 24-hour incubation at $35^0$C. The administration of NaOH solution in the β-hematin pellet will convert it into alkaline hematine whose absorbance can be measured using UV.8 spectrophotometer (Tekwani BL and Walker LA, 2005).

The result of calculation on percentage of β-hematin formation inhibition in table 1 shows that in the group which was given 96% ethanol extract of Sambiloto herbs, β-hematin formation activity was inhibited with the average percentage of β-hematin formation inhibition of 61,07 ± 4,69%.

Frolich et al (2005) suggests that compounds with β-hematin formation inhibition greater than 60% are stated to have good potential as β-hematin inhibitors.

Compounds with β-hematin formation inhibitions smaller than 40% are claimed to be weak inhibitors on the formation of ß-hematin.12 Therefore, it can be stated that 96% ethanol extract of the Sambiloto herbs has good potential as an β-hematin formation inhibitor.

This result is supported by a research conducted by Widyawaruyanti et al (2015) which states that 96% ethanol extract of Sambiloto herbs is known to have activity of inhibiting the process of detoxification of heme into crystalline polymer hemozoin through observation of food vacuoles parasit P.falciparum with TEM (Transmission Electron Microscope) microscope. It was mentioned that, in the parasite which was given 96% ethanol extract Sambiloto herbs, it appears that the crystal hemozoin was undergoing fragmentation.

On the contrary, in the control group, it appears that the woven crystals are still clear and transparent (Widyawaruyanti, 2015).

The aformentioned description shows that ethanol extract of Sambiloto herbs has an inhibitory effect on the detoxification process of heme parasite Plasmodium falciparum and is one of ethanol extract work target of 96% Sambiloto herbs. This inhibition process can minimize the pathogenesis of malaria and also inhibit the formation of hemozoin in the liver and spleen of malaria patients.

Table 1: The percentage of β-hematin formation inhibition by 96% ethanol extract of Sambiloto herbs

| No | Substance | R | Absorbance (414 nm) | % β-hematin formation | % β-hematin inhibition | % mean inhibition of β-hematin |
|---|---|---|---|---|---|---|
| 1 | Control | 1 | 0,73 | 100 | - | - |
| | | 2 | | 100 | - | - |
| | | | 0,71 | | | |
| | | | 0,26 | 35,62 | 64,38 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 2 | 96% ethanol | 1 | | | | 61,07±4,69 |
| | extract | 2 | 0,30 | 42,25 | 57,75 | |

# 4 CONCLUSIONS

In conclusion, 96% ethanol extract of Sambiloto herbs have the activity of inhibiting the process of heme parasite Plasmodium detoxification

# ACKNOWLEDGEMENTS

We thank the staff in Institute of Tropical Disease (ITD) Surabaya for their assistance in preparing a test substance and for help with processing the spectrophotometer sample

# REFERENCES

Bassilico N, Pagani E, Monti D, Olliaro P and Taramelli D, 1998. A microtitre based method for measuring the haem polimerization inhibitory activity (HPIA) of antimalarial drugs. *J. Antimicrob. Chemother*. Vol. 42, pp. 55-60

Egan TJ, 2008. Hemozoin formation. *Journal molecular & biochemical parasitology*. Vol.157, pp. 127-136.

Frolich S, Schuberth C, Bienzle U, Siems KJ, 2005. In vitro antiplasmodial activity of prenylated chalcon derivates and their interaction with haemin. *J. Antimicro Chemother*.Vol. 55, pp. 883-887.

Hong-Chang Z, Yu-hui G, Xiang Z and Heng W, 2009. Dynamin like protein 1 participated in the hemoglobin uptake pathway of *Plasmodium falciparum*. *Chin Med J*. Vol. 122, No. 14, pp. 1686-1691.

Hoppe HC, Schalkwyk DA, Wiehart UIM, Meredith SA, Egan J, Weber BW, 2004. Antimalarial quinolines and artemisinin inhibit endocytosis in *P. falciparum*. *J. antimicrob. Agents Chemother*. Vol. 48, No. 7, pp. 2370-2378.

Okhuarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, Langer P, 2014. Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: a review of its phytochemistry and pharmacology. *Asian Pacific Journal of Tropical Disease*. Vol. 4, No. 3, pp. 213-222.

Rahman N, Furuta T, Kojima S, Takane K, Ali Mohd M, 1999. Antimalarial activity of extracts of Malasyian medicinal Plants. *J ethnopharmacol*.Vol.64, No. 3, pp.249-254

Resi, 2013. Pengaruh pemberian antimalaria herba sambiloto (*Andrographis paniculata* Nees) terhadap perkembangan stadium dan perubahan morfologi parasit Plasmodium falciparum. Tesis. Program Pascasarjana Universitas Airlangga, Surabaya.

Safitri EY, 2007. Penetapan parameter standar simplisia dan ekstrak etanol herba sambiloto (AP). Skripsi. Fakultas Farmasi Universitas Airlangga. Surabaya

Syarif RA, 2007. Aktivitas Antiplasmodium Fraksi Larut Eter Ekstrak Metanol Daun Kembang Bulan (*Tithonia diversifolia* (Hemsley) A. Gray) Pada *Plasmodium falciparum* secara *In vitro*. Tesis. Universitas Gadjah Mada. Yogyakarta

Tekwani BL and Walker LA, 2005. Targetting the hemozoin synthesized pathway for new antimalarial drug discovery: technologies for in vitro ß-hematin formation assay. *Combinatorial Chemistry & High Throughput Screening*. Vol. 8, pp. 63-79

Widyawaruyanti A, 2009. Pengembangan fitofarmaka obat malaria dari fraksi diterpen lakton herba sambiloto (*Andrographolis paniculata* Nees). Lembaga Penelitian Universitas Airlangga. Surabaya.

Widyawaruyanti, Safarianti, Ilmi H, Tumewu L, Hafid AF, 2015. Antimalarial Effects of *Andrographis paniculata Nees* of *Plasmodium falciparum* Food Vacuole. In ISPSA 2015 Tokushima Program & Abstract. Tokushima Bunry University. Japan

Widyawaruyanti, Asrorya M, Ekasaria W, Setiawan D, Radjaram A, Tumewu L, Hafid AF, 2014. In vivo Antimalarial Activity of Andrographis paniculata Tablets. Procedia chemistry. Vol. 13. Pp. 101-104

Widyawaruyanti, Resi EM, Ilmi H, Hafid AF, Tantular IS, 2015. Journal of Indonesian Medical Association. (submiited)