

# Comparative Study of Antimalarial Effect of Sambiloto (*Andrographis paniculata*) Extract, Chloroquine and Artemisinin and Their Combination Against *Plasmodium falciparum* In-vitro

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## ABSTRAK

**Tujuan:** untuk membandingkan efek antimalaria antara ekstrak sambiloto, chloroquine dan artemisinin, dan juga kombinasinya. **Metode:** penelitian dilakukan di Laboratorium Sentral Biomedik Fakultas Kedokteran Universitas Brawijaya Malang Indonesia, mulai Januari sampai Februari 2006. Kultur malaria menggunakan *Plasmodium falciparum* strain Papua (2300) yang berasal dari Namru-2 Jakarta. Lima kelompok obat yang diuji yaitu, chloroquine, artemisinin, ekstrak sambiloto, kombinasi sambiloto dan chloroquine, dan kombinasi sambiloto dan artemisinin. Kepadatan parasit ditentukan dengan menghitung jumlah *Plasmodium falciparum* yang menginfeksi eritrosit pada 5.000 kultur eritrosit. Efek membunuh parasit obat tunggal (Chloroquine saja atau artemisinin saja) dan kombinasi dengan sambiloto pada dosis 0,5 ug/ml dinilai dengan terlihatnya gambaran "crisis form" pada eritrosit yang terinfeksi. **Hasil:** ekstrak sambiloto pada dosis 0.5 ug/ml meningkatkan kepadatan parasit dan pada peningkatan dosis 1 ug dan selanjutnya efek membunuhnya meningkat. Penurunan kepadatan parasit juga terlihat dengan peningkatan dosis pada kelompok kombinasi sambiloto-chloroquine dan sambiloto-artemisinin. Secara statistik, tidak ada perbedaan yang bermakna efikasi antimalaria antara kelima kelompok uji ( $p=1,00$ ). Korelasi antara penurunan kepadatan parasit dengan peningkatan dosis pada semua kelompok obat uji secara statistik signifikan ( $p=0,001$ ). **Kesimpulan:** ekstrak sambiloto tunggal atau kombinasi terbukti mempunyai efek antimalaria *falciparum*.

**Kata kunci:** ekstrak Sambiloto, efek antimalaria, uji in-vitro.

## ABSTRACT

**Aim:** to compare the anti-malarial effect among sambiloto extract, chloroquine and artemisinin-only as well as those of their combination. **Methods:** the study was conducted in Central Biomedical Laboratory, Faculty of Medicine, Brawijaya University, Malang, Indonesia from January to February 2006. Malaria culture used *Plasmodium falciparum* of Papua strain (2300) that was obtained from Namru-2 Jakarta. Five drugs applied in this test; those were chloroquine, artemisinin, the extract of sambiloto, the combination of sambiloto and chloroquine, and the combination of sambiloto and artemisinin. Parasite density was determined by counting the number of *Plasmodium falciparum* infected erythrocyte in 5,000 erythrocytes of the culture. Single drug (Chloroquine-only or artemisinin only) and either combination with sambiloto at dose 0.5 ug/ml had killing-effect against the parasite, measured by the appearance of "crisis form" on the infected erythrocytes. This killing-effect was dose dependent, and reached its optimum effect of 200 ug/ml. **Results:** treatment of single sambiloto extract with dose 0.5 ug/ml increased the density of the parasite, however after every 1ug increasing dose of sambiloto extract, the killing effect also increased. The reduction of the parasite density was also seen

by increasing the Sambiloto dose in the group of combination of sambiloto-chloroquine as well as the group of combination of sambiloto and artemisinin. Statistically, there was no difference in the anti-malaria efficacy among of five test drugs ( $p=1.00$ ). The correlation between the reduction of the parasite with the increasing of dose in all groups is statistically significance ( $p=0.001$ ). **Conclusion:** the extract of sambiloto in a single dose or in a combination evidently has the effect of anti-falciparum malaria.

**Key words:** extract of sambiloto, antimalaria effect, in-vitro test.

## INTRODUCTION

Sambiloto herb (*Andrographis paniculata* nees) is one of medicinal plants available over Indonesia with various local names. Javanese has already known this multi branched-bush as a potent drug for snake poisons since old time.<sup>1</sup> Sambiloto which is also known as “King of Bitters”, is a plant from family *Acanthaceae* that has been used for centuries in Asia to treat digestive and respiratory diseases, fever, herpes, throat infection, and many other chronic and infection diseases, including malaria.<sup>2</sup> Its components exhibit an anti-inflammatory, anti-carcinogenic, anti-bacterial, anti fertility, anti malaria, anti HIV, anti diarrhoeal and intestinal effect, anti diabetic, and have hepatoprotective and cardiovascular activity.<sup>3,4</sup> Its major active phytochemical have been extensively studied for anti oxidant, anti venom, anti leishmanial, anti filaricidal, anti microbial and anti fungal.<sup>4</sup>

Two new flavonoid glycosides, 5-hydroxy-7,8-dimethoxy (2R)-flavanone-5-O-beta-D-glucopyranoside and 5-hydroxy-7,8,2',5'-tetramethoxy-flavone-5-O-beta-D-glucopyranoside, and a new diterpenoid, andrographic acid, along with andrographidine were isolated from *A. paniculata*, and their structures were determined on the basis of physicochemical and spectroscopic analysis.<sup>5</sup> Four xanthenes are reported from the roots of *A. paniculata*. The primary bioactive component of *A. paniculata* is andrographolide.<sup>3,4</sup> Andrographolide was responsible for anti hepatotoxic, anti allergic, anti cancer, anti inflammatory, antimicrobial activities, larvicidal, ovidical and testicular toxicity, while xanthenes showed anti plasmodial activities.<sup>6</sup>

*Andrographis paniculata* (kalmegh), has been studied in a four day suppressive test against *Plasmodium berghei* NK 65 in *Mastomys natalensis*. A crude ethanol extract and fractions reduced the level of parasitaemia in a dose-dependent manner.<sup>7</sup> Until now there is no research

regarding anti-malaria effect of Sambiloto (*A. paniculata*) in-vitro. Therefore, this study was done to analyze the difference in anti-malarial effect among sambiloto extract, chloroquine, artemisinin, sambiloto extract in combination with Chloroquine or with artemisinin in-vitro using *Plasmodium falciparum* 2300 (Papua strain).

## METHODS

The study was conducted in The Central Biomedical Laboratory of Faculty Medicine, Brawijaya University starting January through February 2006. Malaria culture used, *Plasmodium falciparum* of Papua strain (2300) was originated from Namru-2 Jakarta.

Growth inhibition was determined using different concentrations of Sambiloto extracts on synchronized *P. falciparum* cultures at the ring stage. Five drug groups were applied in this test; those were chloroquine group, artemisinin group, the extract of sambiloto group, the combination of sambiloto and chloroquine group, and the combination of sambiloto and artemisinin group. Parasite density was determined by counting the number of *Plasmodium falciparum* in 5,000 erythrocytes of the culture. Concentration of Sambiloto extract, chloroquine and artemisinin are 0,5 ug/ml, 1 ug/ml, 5 ug/ml, 10 ug/ml, 50 ug/ml, 100 ug/ml, 200 ug/ml respectively both in single and combination treatment.

### Preparation of *A. paniculata* Stem Extract

Sambiloto extract preparation is done in laboratory of Faculty of Pharmacy Sumatera Utara University Medan, Indonesia. As much as 8.4 kg powder simplisia inserted into a closed tube and colourless with solvent ethanol 50%, and the process of maceration for three hours. The masses were transferred little by little into the percolator, then sparingly until the solvent is poured on top of a liquid simplisia solvent still contained, percolator closed and left for 24 hours. The liquid was left dripping with a speed of 1 ml

per minute, the solvent is added continuously until the solvent layer occurs above simplisia. Percolation process was stopped until the last out does not percolate taste bitter. Percolate was evaporated by using a vaporizer vacuum play up to thick extract is obtained.

**Preparation of Drug Solution (Artemisinin, Chloroquine, Extract the Stem *A. paniculata*)**

Artemisinin was made into solution with a dose of 0.5 ug/ml, 1 ug/ml, 5 ug/ml, 10 ug/ml, 50 ug/ml, 100 ug/ml by dissolving artemisinin in 70% ethanol, the next dilution used RPMI 1640 medium and the last using complete medium 10% as diluting agent. Extracts of stem *A. paniculata* was dilute with similar method to those of Artemisinin. Chloroquine was made to be a solution with a concentration of 0.5 ug/ml, 1 ug/ml, 5ug/ml, 10 ug/ml, 50 ug/ml, 100 ug/ml 200 ug/ml by dissolving it in aquadest, the dilution was added with RPMI 1640 and then use complete medium 10% as diluting agent.

**Antimalarial Activity of *A. paniculata* Extract Compared with Standard Drugs (Artemisinin and Chloroquine) in-vitro**

Frozen *P. falciparum* 2300 was thawed and cultivated in a culture medium containing blood group O erythrocytes with a hematocrit of 5% and the human blood type AB serum 10% in the incubator with stream of CO2 5% at 37°C. Culture medium was replaced every day and cultivated to obtain the level of parasitemia >5%. To test the antimalarial activity of *A. paniculata* extract, artemisinin and chloroquine, we put a volume of 100µl synchronized culture medium consist of *A. paniculata* extracts with dose of 0,5 ug/ml, 1 ug/ml, 5 ug/ml, 10 ug/ml, 50 ug/ml, 100 ug/ml 200 ug/ml in sterile micro-plates consisting of 96 wells, each well containing 100 ul malaria culture pellet and incubated for 48 hours. In the same time we put a volume of 100µl culture medium containing artemisinin as well as chloroquine with similar dose of *A. paniculata* extract in sterile micro-plates

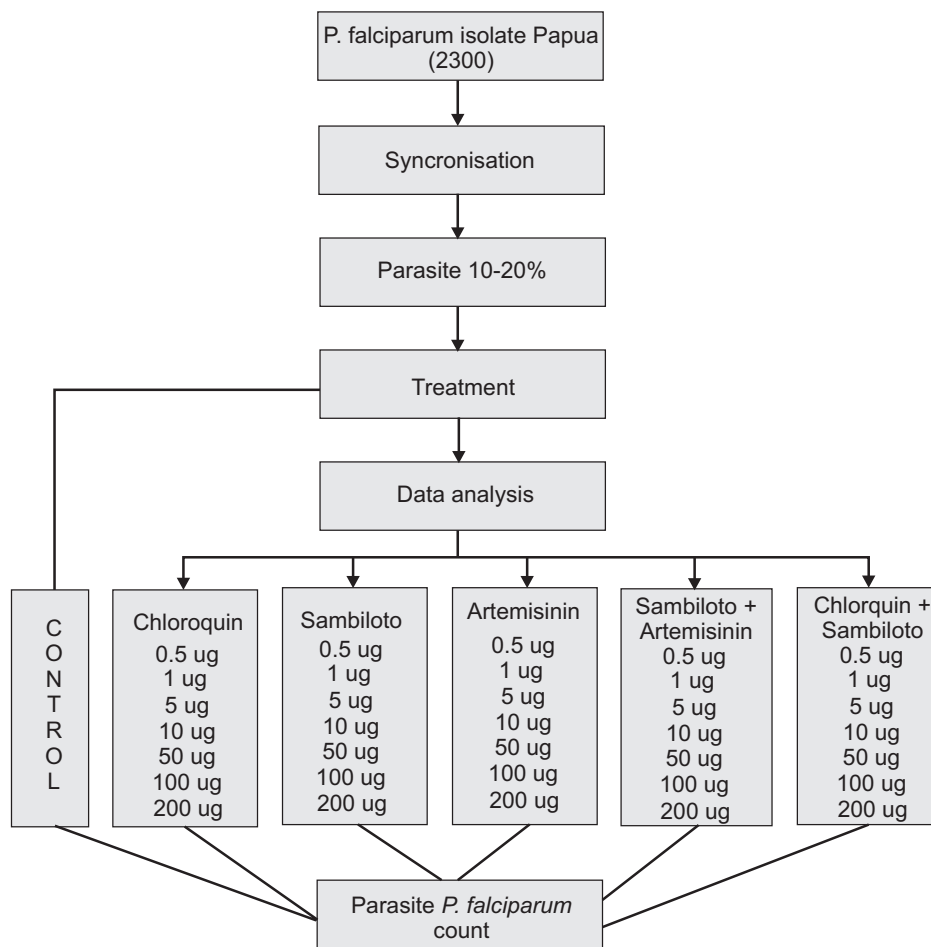


Figure 1. In-vitro test study design

consisting of 96 wells, each well containing 100 ul malaria culture pellet and incubated for 48 hours. We conducted in the same procedure to test antimalarial activity of combination of Sambiloto and Chloroquine and Sambiloto and Artemisinin. After 48 hours, a thin blood smear was made and examined microscopically by Giemsa staining. Performed counting the number of infected erythrocytes in 5000 erythrocytes, then the number of erythrocytes was used as a percentage of parasite/100 erythrocytes. We used wells with a volume of 200 ul complete medium as control group. For control and each drug group a triplicate wells have been used.

Statistical analysis by Kolmogorov-Smirnov test, and we found that distribution of data is abnormal and continued by Kruskal-Wallis analysis.

## RESULTS

Single drug (Chloroquine-only or artemisinin only) and either combination with sambiloto have the killing-effect against the parasite at the dose of 0,5 ug/ml, and, this killing-effect increase simultaneous with the increasing of the Sambiloto dose applied which reached its optimum dose of 200 ug/ml. Interestingly for the group of Sambiloto-only, the density of the parasite for the 0.5 ug/ml-dose was higher than the control group, but for every raising of 1 ug/ml of Sambiloto, the killing effect would increase and continue to raise with the increase of the dose. For the group of sambiloto-artemisinin combination, the decline of the parasite density was also occurred by the increased dose of the test drug (Table 1). For the group of Sambiloto-Chloroquine combination, the reduction of the parasite density was also seen by increasing the

drug dose, and statistically, there is no difference with the efficacy of five test drugs at  $p = 1$ , and the reduction of the parasite with the increasing of dose in all groups is statistically high significance ( $p < 0,001$ ).

Figure 2–5 showed parasite density in control and some drugs test in 0.5 ug/ml and 200 ug/ml doses. Figure 6 showed the correlation between the reduction of the parasite with the increasing of dose in all groups is statistically significance ( $p = 0.001$ ).

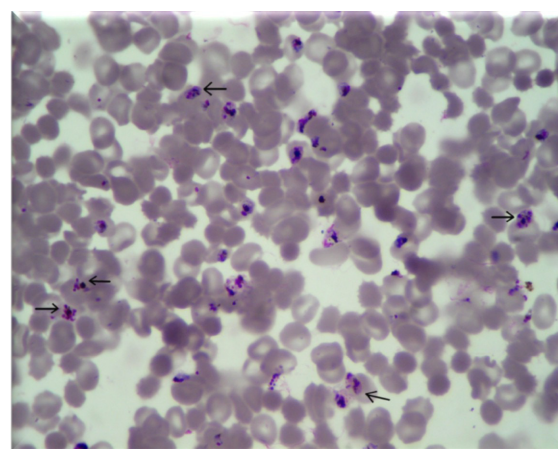


Figure 2. Parasite density in *P. falciparum* culture (control)

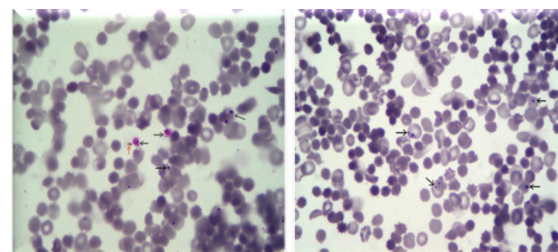


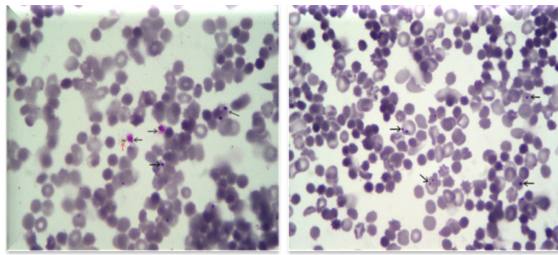
Figure 3. Parasite density in *P. falciparum* culture with Chloroquine doses 0,5 ug and 200 ug

Table 1. The reduction of *Plasmodium falciparum* density by increasing the drug

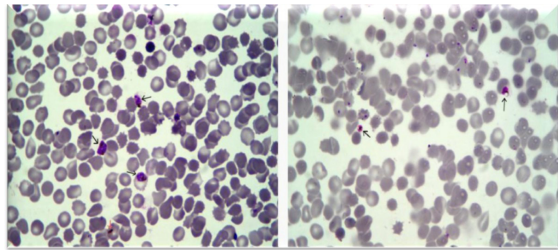
Test Group	Parasite Density (%)							
	Control	0,5 ug	1 ug	5 ug	10 ug	50 ug	100 ug	200 ug
Chloroquin	41	17,93	15,43	11,17	8,83	6,6	5,47	5,27
Sambiloto	38,17	47,33	41,33	31,33	27,53	21,5	13,33	10
Artemisinin	38,08	18	13,23	10,27	8,67	7,58	5,93	4,13
Arte+Sam.	44,73	29,47	22,67	19	17,67	17,67	12,33	10
Chloro+Sam	30,2	21,27	17,53	13,3	10,27	6,8	5,73	23

Arte=artemisinin, Sam=sambiloto, Chloro=chloroquine





**Figure 4.** Parasite density in *P. falciparum* culture with Artemisinin doses 0,5 µg and 200 µg



**Figure 5.** Parasite density in *P. falciparum* culture with Sambiloto extract doses 0,5 µg and 200 µg

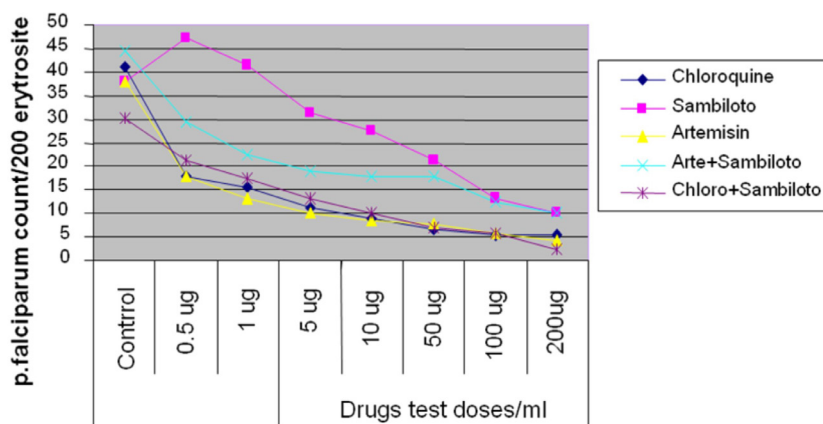
**DISCUSSION**

In this study a low dose of Sambiloto (0.5 ug/ml) can increase the proliferation of parasite, it was revealed by the higher parasite density than the control. We suggest that at low dose Sambiloto will acts as anti-oxidant drug that will scavenge a variety of free radicals that very harmful to the parasite. Several literarure studies that based on research studies have been showed a potent anti-oxidant effect of andrographolide. Andrographolide is a potent scavenger of Reactive Oxygent

Species (ROS) including superoxide anion, hydroxyl radicals, singlet oxygen, peroxinitrit and nitric oxide.<sup>3,4</sup> The downregulation of ROS production via a protein kinase-C (PKC) dependent mecahnism and via prevention of de novo protein synthesis or decreasing the protein stability in post-transcriptial mecahnism.<sup>3</sup> Andrographolide also causes a significant elevation of catalase, superoxide dismutase and glutathione-s-transferase activities.<sup>4</sup> The presence of 14-deoxy andrographolide and 14-deoxy 11,12 dihydroandrographolide cause anti-oxidant activities.<sup>6</sup> However with the increasing of Sambiloto extract dose, the anti oxidant effect change to anti malaria property.

This study showed that a combination of Sambiloto extract with both chloroquine and artemisinin showed similar effect in their anti-malarial activity. In-vitro potency was observed with the combination of Sambiloto extract with chloroquine and artemisinin similar to the individual extracts. Sambiloto extract was found to inhibit the ring stage of the parasite whether used in isolation or in combination. The deciphering of anti-malarial activity in andrographolide against the blood stage of the plasmodial life cycle lays foundation to reevaluate its possible role in the regulation of this important transcription factor for the effective control of malaria.

Recent study assessed the anti-malarial properties of two Indian traditional plants, *Andrographis paniculata* and *Hedyotis corymbosa*, compared their activity individually



**Figure 6.** Decreasing of *P. falciparum* density with increasing drugs test doses in-vitro. The correlation between the reduction of the parasite with the increasing of dose in all groups is statistically significance (p=0.001).

and in combinations with curcumin had been conducted in mice infected with *Plasmodium berghei*. The result showed that the 50% inhibitory concentration (IC50) of *A. paniculata* better than *H. corymbosa* combination of this two herbal and combination of each herbal with chloroquine enhanced their anti-malaria activity.<sup>8</sup>

Andrographolic, the diterpene lactone compound that isolated by HPLC from methanolic fraction of *A. paniculata* was found to have potent anti-malaria activity and synergistic potency with curcumin and additively interactive with artesunate. In mice that infected with *Plasmodium berghei*, andrographolide-curcumin exhibited better antimalarial activity, not only by reducing parasitemia (29%), compared to the control (81%), but also by extending the life span by 2-3 folds.<sup>8</sup>

This study showed that both combination of Sambiloto extract with artemisinin and with Chloroquine did not increase the effectiveness of antimalaria effect. This result is similar to the recent research that used chloroquine resistant strain of *Plasmodium falciparum* (MRC-pf-303) in vitro. A combination of andrographolide-artesunate was found effective only at lower ratio.<sup>9</sup> A different result come from our clinical study for malaria patients that treated by Sambiloto herbs in simplicia capsule. Administration of 250 mg Sambiloto herbs in simplicia capsule increase sensitivity level of *Plasmodium*. Resistance value is 48% and 12.5% of *Plasmodium falciparum* to chloroquine and chloroquine-sambiloto, respectively.<sup>10</sup>

## CONCLUSION

The extract of Sambiloto evidently has the effect of anti-falciparum malaria in-vitro and a maximum effect at a dose of 200 ug/ml. The effect of extract of Sambiloto as antimalaria had been revealed both in a monotherapy and in a combination.

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