

Chemical Constituent and Antimalarial Activity based on Inhibition of Heme Polymerization from Water Extract of Yellow Root (*Arcangelisia flava* L. Merr)

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Abstract: In developing countries, malaria remains a disease that can spread easily and caused death. Malaria is an infectious disease caused by *Plasmodium sp* involving female anopheles masquitos during its transmission. *Arcangelisia flava* L. Merr has been investigated earlier that it can inhibited *P. falciparum* growth. Method of antimalarial activity based on inhibition of heme polymerization can confirm one of the mechanism of antimalarial drugs. The aim of this research to study further antimalarial activity and IC₅₀ based on inhibition of heme polymerization and determine the chemical constituent from water extract of *A. flava* L. Merr. This research was conducted through several steps, namely 1) water extraction, 2) column chromatography (SiO₂ ; (i) CH₂Cl₂-MeOH=10:1~1:1 (ii) *n*-hexane: EA=1:1; CH₂Cl₂-MeOH=10:1~1:1), 3) antimalarial assay, 4) identification of chemical constituent using FTIR and GC-MS. Results of this research are water extract of *A. flava* L. Merr has IC₅₀ 601 ppm and identification of chemical constituent using FTIR and GC-MS was assumed as stigmastan.

Keywords: *Arcangelisia flava*, antimalarial, malaria, heme polymerisation

Abstrak: Malaria tetap menjadi penyakit yang mudah menyebar dan menyebabkan kematian di negara-negara berkembang. Malaria merupakan penyakit menular yang disebabkan oleh *Plasmodium sp* yang disebarkan oleh nyamuk *Anopheles betina*. *Arcangelisia flava* L. Merr telah ditemukan memiliki kemampuan menghambat pertumbuhan *Plasmodium falciparum*. Metode aktivitas antimalaria berdasarkan penghambatan polimerisasi heme dapat mengonfirmasi salah satu mekanisme obat antimalaria. Tujuan dari penelitian ini adalah mempelajari lebih jauh aktivitas antimalaria dan IC₅₀ berdasarkan penghambatan polimerisasi heme dan menentukan komponen senyawa kimia dari ekstrak air *A. flava* L. Merr. Penelitian ini dilakukan melalui beberapa tahap, yaitu 1) ekstraksi dengan air, 2) kromatografi kolom (SiO₂ ; (i) CH₂Cl₂-MeOH=10:1~1:1 (ii) *n*-hexane: EA=1:1; CH₂Cl₂-MeOH=10:1~1:1), 3) uji antimalaria, 4) identifikasi senyawa kimia dengan FTIR dan GC-MS. Hasil penelitian menunjukkan bahwa ekstrak air memiliki nilai IC₅₀ sebesar 610 ppm dan identifikasi senyawa kimia menggunakan FTIR dan GC-MS menunjukkan senyawa aktif adalah stigmastan.

Kata kunci: *Arcangelisia flava*, antimalaria, malaria, polimerisasi heme

INTRODUCTION

Malaria is one of the most important parasitic diseases and is widely endemic in tropical, subtropical, and temperate regions (Cotter *et al.* 2013). There are currently over 100 countries and territories where there is a risk of malaria transmission. The most severe form is caused by *P. falciparum*; variable clinical features include fever, chills, headache, muscular aching and weakness, vomiting, cough, diarrhea and abdominal pain.

Malaria is one of the public health problems that can cause death especially in high risk group that is infant, toddler, pregnant mother, besides malaria directly cause anemia and can decrease work productivity. The disease is also still endemic in most parts of Indonesia (Kementerian Kesehatan RI 2011). Updated estimates have indicated that 212 million cases occurred globally in 2015, leading to 429,000 deaths, most of which were in children under 5 years of age in Africa (WHO 2016). Although great

success has been achieved since the launch of the national malaria control programme in 1955, malaria remains a serious public health problem in China (Tang 2000), where *Plasmodium vivax* and *Plasmodium falciparum* have historically been present at high frequencies (Zhang *et al.* 2014).

The levels of *P. vivax* endemicity vary widely among the World Health Organization (WHO) regions. Outside of Africa, *P. vivax* is the dominant species, with a relatively high prevalence of infection in the Southeast Asian and Western Pacific regions (WHO 2015). Furthermore, *P. vivax* and *P. falciparum* were the predominant species in countries in the preelimination and elimination phases, which had low total annual malaria incidence rates (Feachem 2010). Other symptoms related to organ failure may supervene, such as acute renal failure, pulmonary oedema, generalized convulsions, circulatory collapse, followed by coma and death. The initial symptoms, which may be mild, may not be easy to recognize as being due to malaria. Despite continuous global attempts to fight parasitic infections, malaria still remains one of the major human life-threatening diseases. Difficulty of producing efficient antimalarial vaccines and increasing drug-resistant strains, highlight the urgent need to search for a new alternative antimalarial drug. *Kayu kuning* is one of the medicinal plants that growth in the Meru Betiri National Park. *Kayu kuning* has been used as a traditional medicine to treat malaria, dysentery, and fever in Kalimantan (Subeki *et al.* 2004). Isolation of tryacontanyl caffeat from *Kayu kuning* trunk shown potential as an antioxidant (Keawpradub 2005).

Arcangelisia flava (L.) Merr. has been used as one of traditional medicines (Jamu) in Indonesia. The medicinal plant is known locally as 'Akar kuning', the name derived from the yellow sap. *Fibraurea tinctoria* Lour. and *Cosinium fenestratum* Colebr. were also called 'Akar kuning' in Indonesia. The genus *Arcangelisia* (Menispermaceae) comprises two liana species (Forman 1978). The shoot and root decoctions of *A. flava* are used locally for a variety of purposes: febrifuge, tonic, abortive, and the healing of hepatitis, indigestion and malaria (Keawpradub *et al.* 2005; Nguyen-Pouplin *et al.* 2007). Recent pharmacological investigations have shown that the extracts have diverse biological effects such as antimicrobial, antibabesial, cardiogenic and anti-hypertensive (Subeki *et al.* 2005). *A. flava* L. Merr has been investigated earlier that it can inhibit *P. falciparum* growth. Malaria parasite has several unique pathways that can be explore in order to find antimalarial drugs. One of antimalarial mechanism that use as finding new antimalarial drug is inhibition of hemozoin polymerisation. Despite chloroquine resistance, hemozoin is still a fascinating valid drug target. β -hematin, a structurally identical to hemozoin is used to study inhibition of hemozoin polymerisation *in vitro* (Sandlin 2013). Antimalarial

activity based on inhibition of heme polymerization can confirm one of the mechanisms of antimalarial drugs.

MATERIALS AND METHODS

Materials

The materials used in this study were water extract of *A. flava*, hematin, dimethylsulfoxide, dichloromethane, chloroform, methanol, 0.1 M sodium hydroxide, glacial acetic acid, chloroquin sulphate, aquadest.

Instruments

The instruments used in this study were water bath, Hitachi Microcentrifuge CS 150 NX, Fourier Transform Infra Red (Shimadzu 8400 S), Gas Chromatography Mass Spectroscopy (Agilent Technologies 5973 inert, 6890 N),

Extraction

As much as 250 grams of dried *A. flava* was extracted using boiling water, repeated 3 times. Water extract was concentrated using water bath.

Column Chromatography

Water extract of *A. flava* was fractionated using column chromatography with gradient method. Stationary phase was silica gel and gradient mobile phase were dichloromethane : methanol (10:1-1:1). Fractions were collected based on thin layer chromatogram.

Antimalarial Activity Assay

Antimalarial activity were tested to fractions based on inhibition of heme polymerisation with modification (Basilico *et al.* 1998). One hundred microlitres of a 0.5 mM solution of haematin, previously dissolved in 0.1 M NaOH, were distributed in 96-well U-bottomed microplates. Fifty microlitres of different doses of antimalarial drugs. Added to triplicate test wells. Either 50 μ L of water or 50 μ L of the solvent used to solubilize the drugs were added to control wells. Haematin polymerization was initiated by adding 50 μ L glacial acetic acid pH of 2.6 and the suspension was incubated at 37°C for 24 h to allow complete polymerization. Plates were then centrifuged at 8000 rpm for 15 min and the soluble fraction of unprecipitated material collected (fraction I). The remaining pellet was resuspended with 200 μ L of DMSO to remove unreacted haematin. Plates were then centrifuged again at 8000 rpm for 15 min. The DMSO-soluble fraction (fraction II) was collected and the pellet, consisting of a pure precipitate of haematin, was dissolved in 0.1 M NaOH (fraction III) for spectroscopic quantification. A 150 μ L aliquot of each fraction was transferred on to a new plate and serial four-fold dilutions in 0.1 M NaOH were performed. The amount of haematin was determined by measuring the absorbance at 405 nm using a

microtitre plate reader. A standard curve for haematin dissolved in 0.1 M NaOH was used to calculate the amount of porphyrin present in each fraction (Basilico *et al.* 1998).

The highest antimalarial activity was fractionated using column chromatography. IC₅₀ of water extract of *A. flava* was tested and compared to chloroquine sulfate as positive control.

Identification of Chemical Constituents using FTIR and GC-MS

Fraction that has highest antimalarial activity among other fractions were fractionated using preparative TLC. Band from preparative TLC were collected and analyzed with FTIR and GC-MS.

RESULTS AND DISCUSSIONS

Extraction

From 250 gram of dried *A. flava* that was extracted with aquadest 24.28 gram of extract was obtained. Yield from water extraction is 9.71%. Water was selected as solvent because local people usually used bark of *A. flava* by boiling with water and drink it as medicine.

Antimalarial Activity of Fractions from First Column Chromatography

Water extract of *A. flava* was homogenized with cellite to purify using column chromatography. Silica gel was used as stationary phase and gradient dichlorometane-methanol (10:1~1:1) was used as mobile phase. Fractions were collected from column chromatography and differentiated based on TLC pattern. Six fractions were tested for antimalarial activity and results is showed in Table 1.

Table 1. Percent of inhibition of heme polymerisation from first column chromatography fractions

Fractions	Concentration (μM)	Percent of Inhibition (%)
1	142.50	56.54
2	151.65	42.66
3	103.65	62.99
4	185.93	29.26
5	193.91	20
6	176.98	31.13

Fraction 3 with the highest antimalarial activity was purified further using column chromatography. *Plasmodium* sp degrades hemoglobin in erythrocytes to feed and releases free heme which toxic to host cells and the plasmodium itself. In order to protect

itself, *Plasmodium* detoxifies free heme into: neutralize with histidine-rich protein, degradation with reduced glutathione or crystallization into hemozoin. Hemozoin is widely accepted as the main pathway of heme detoxification in the parasite (Nhien *et al.* 2011).

Beta hematin (BH) has analogue structure to hemozoin. Recent reports showed that inhibiting formation of BH is an ideal target for antimalarial screening (Huy *et al.* 2007). This method will confirm antimalarial mechanism through inhibition of heme polymerisation.

Antimalarial Activity of Fractions from Second Column Chromatography

Fraction with highest antimalarial activity was fractionated further with column chromatography. Silica gel was used as stationary phase and *n*-hexane-ethyl acetate (1:1) and dichlorometane-methanol (10:1~1:1) sequentially were used as mobile phase. Fractions were collected from column chromatography and differentiated based on TLC pattern. Four fractions were tested antimalarial activity and result was showed in Table 2.

Table 2. Percent of inhibition of heme polymerisation from second column chromatography fractions

Fractions	Concentration (μM)	Percent of Inhibition (%)
1	39.40	72.63
2	92.68	20.01
3	31.39	78.66
4	46.26	68.55

Fraction 3 showed the highest antimalarial activity which higher than fraction before fractionated with column chromatography. Due to limitation amount of fraction, it can not undergo column chromatography. In order to purify fraction 3 of second column chromatography, preparative TLC was chosen to simplify compound from fraction 3.

IC₅₀ value of water extract *A. flava* and Chloroquine Sulfate

IC₅₀ value of water extract of *A. flava* (Table 3) was compared to chloroquine sulfate (Table 4) as positive control. Water extract of *A. flava* has higher antimalarial activity than chloroquine sulfate.

Chloroquine sulfate as positive control also known as antimalarial drug was showed inhibit the formation of synthetic heme crystal, β-hematin (BH) which is structurally identical to hemozoin (Huy *et al.* 2007).

Table 3. IC₅₀ value of water extract *A. flava*

	Concentration (mg/mL)	Percent of inhibition (%)	IC ₅₀ (ppm)
Water extract <i>A. flava</i>	2	76.22	601
	1	58.65	
	0.5	41.09	
	0.25	33.71	
	0.125	22.83	

Table 4. IC₅₀ value of chloroquine sulfate

	Concentration (mg/mL)	Percent of inhibition (%)	IC ₅₀ (ppm)
Chloroquine Sulfate	1	56,19	916,64
	0,5	39,34	
	0,25	29,86	
	0,125	14,75	
	0,0625	10,18	
	0,03125	7,02	

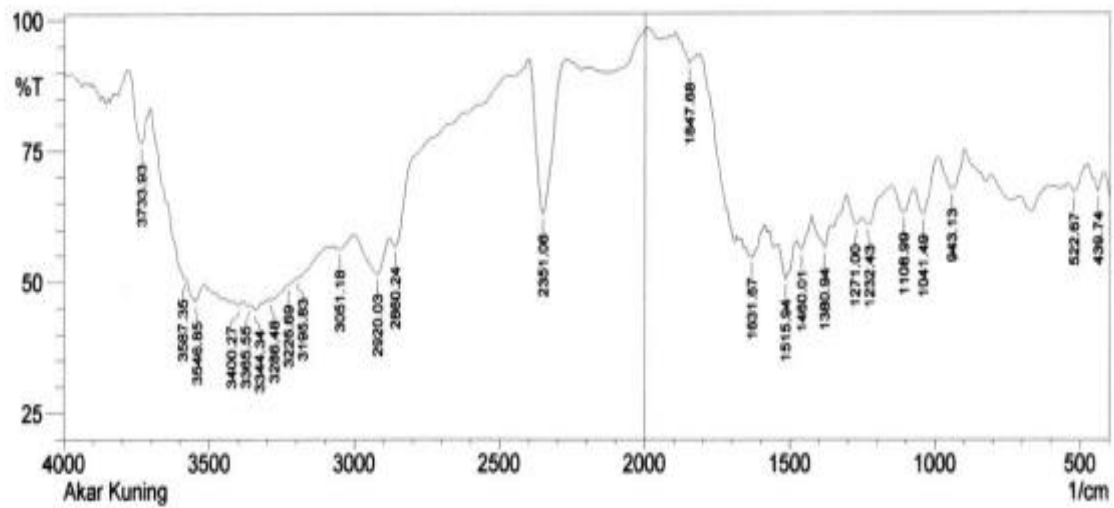


Figure 1. Fourier transform infra red spectra of isolate of *A. flava* extract

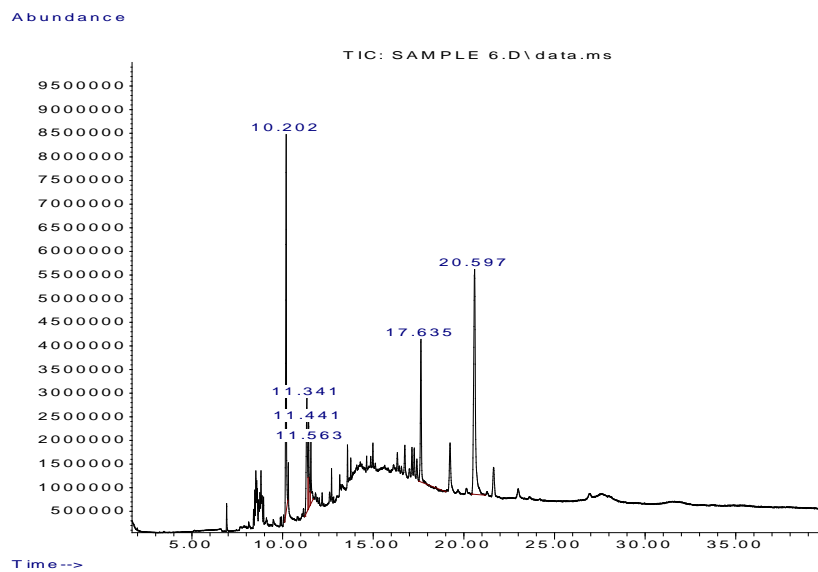


Figure 2. Gas chromatography mass spectroscopy spectrum of isolate of *A. flava* extract

Identification of Chemical Constituent using FTIR and GC-MS

Fraction with highest antimalarial activity was purified using preparative TLC. Band was separated and collected to analyse using FTIR and GC-MS. FTIR spectra was showed in Figure 1. Functional groups from isolated preparative TLC can be analysed using FTIR spectroscopy based on wavelength and frequency.

Based on Figure 1, the isolated compound has nitro compound at wavelength 1515.94 cm^{-1} , also showed functional group of C-O at 1108.99 , 1232.43 , 1271 cm^{-1} which indicated alcohol, eter, ester and carboxylic acid. Amine and amide groups at 3344.34 and 3400.27 cm^{-1} respectively. Alkene groups at 1631.67 cm^{-1} , alkane groups at 2860.24 , 1380.94 and 2920.03 cm^{-1} and hydroxyl group at 3587.35 cm^{-1} (Skoog *et al.* 2007).

GC-MS analysis showed several peaks, this indicated that isolated fraction using prepative TLC is not pure. There were some impurities from contaminants. Based on GC-MS spectra, similarity more than 90% which 93, 96,99 and 97% (Willey09th database) are *p*-nonylphenol, stigmastan-3-5-diene, ergost-5-en-3-ol and stigmastan-5-en-3-ol respectively. Stigmastan is in triterpenoid classes. Stigmastan itself is the most prevalent in the plant kingdom as steroid classes (Civjan 2012). Chemical constituent from isolated *A. flava* which has antimalarial activity was assumed as stigmastan-5-en-3-ol.

CONCLUSIONS

Water extract of *A. flava* has antimalarial activity based on inhibition of heme polymerisation with IC_{50} value 601 ppm meanwhile chloroquin sulphate as positive control was 916.64 ppm. Chemical constituent that fractionated using column chromatography and preparative TLC which analysed using FTIR and GCMS was assumed as stigmastan compound.

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