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In vitro study of parasitemia determination of alkaloids from *S. tuberosa* Lour by flow cytometry in comparation with optical microscopy

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ABSTRACT

Parasitemia is the quantitative content of parasites in the red blood cells, its indication of level of an active parasitic infection in human. Among the methods have been used for counting number of Plasmodium are optical microscopy (OM) and flow cytometry (FCM).The thin blood smear is stained by Giemsa for OM and DNA fluorescent stained detection for FCM. Croomine, epi-croomine and tuberostemonine are alkaloids isolated from the roots of Stemona tuberosa. The parasitemia of their alkaloids were determined in vitro using their methods above at 0.01; 0.1; 1 and 10 ppm concentrations, respectively. The fluorescent for DNA stain is used propidium iodide. The statistical analysis showed that the parasitemia determination on the two methods were almost similar.

Keywords: Parasitemia, alkalois, *Stemona*, optical microscopy, flow cytometry

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INTRODUCTION

Plasmodium infection is one serious of health problems in the world. Located in tropical regions, Indonesia is facing a high risk of malarial endemic [1].The level of parasitic infection can be measured by counting *Plasmodium* content in the human red blood cell (RBC) which is used in anti-malarial activity screening of new drugs through in vitro culture. Commonly, the *Plasmodium* in the RBC was counted by optical microscopy (OM) and flow cytometry (FCM). The first method, Giemsa stain (methylene blue and eosin) was applied on thin blood smear slide and the number of parasite were counted per 1000 RBC. The OM method is still currently being used due to its ability to distinguish five *Plasmodium* and their stages. The FCM method uses fluorescence DNA staining agent and laser aid *Plasmodium* level quantification. Several common dyes are including propidium iodide [11, 12], acridine orange [13, 14], Hoechst 33258 [5, 8, 15], Hoechst 33342 [16, 17], Thiazoleorange [5, 8, 15] hydroethidine [14], cyanin DiOCl [5], Picogreen [10, 15], SYBR Green [7, 18], DRAQ-5 [3, 18], YOYO-1 [14], and dodecyl methyl ammonium chloride [13].

The fact of anti-malarial resistance has emerged for the need of new drugs. For Indigenous people of Indonesia, medicinal plants have been played an important role as a source for anti-malarial agents [19]. This includes the uses of *S. tuberosa* Lour in malarial fever therapy in which our previous research on the root of *S. tuberosa* Lour has revealed potent anti-malarial constituents, croomine and *epi*-croomine. The alkaloids showed competitive inhibition against human dihydrofolate reductase (DHFR) recombinant [20]. Following this positive results, in this paper we present their *in vitro* parasitemia determination against *P. falciparum* 3D7 strain by OM and FCM.

MATERIALS AND METHODS

Alkaloids
The *epi*-croomine, croomine and tuberostemonine were isolated from *S. tuberosa* Lour and their molecular structures were determined through spectrometric and spectroscopic spectral analysis [21].

Phosphat Buffer Saline (PBS)
Mixture NaCl:Na_2HPO_4:KH_2PO_4:KCl (8:1.44:0.24:0.2 g) was dissolved in de-ionize water and made up to 1000 mL. The solution was then adjusted at pH 7,4 using 5N HCl.

Red Blood Cell 50%
Ficoll-Hipaque (d=1.077 g/mL, 5 mL) was mixed and homogenized with blood O (5 mL) in centrifuge tube (15 mL). To a portion of the mixture (5 mL), phosphate buffer saline (PBS) (5 mL) was added and centrifuged at 700 rpm for 30 mins. The erythrocyte layers was separated and washed with PBS (10 mL) and centrifuged at 1000 rpm for 10 mins. This washing procedure was repeated 3 times. Serum free medium was then added at the same volume. A half of RBC was kept in refrigerator at 4^{o}C.

Human Serum O blood
Human serum O bloods were collected in no additive vacutainer at room temperature for 2 hours. The bloods were centrifuged at 1000 – 2000 for 15 mins and the sera were then separated from erythrocyte. Sera were heated at 56^{o}C for 60 mins and was then cooled at the room temperature and kept at -40^{o}C.

Medium
Plus medium (serum free medium): mixture solutions of 10.4 RPMI-1640 (GIBCO) with L-glutamine without $NaHCO_3$, Na_2CO_3 (2.0 g) and hepes (2.8 g) in de-ionized water (1000 mL). To a portion of plus medium (100 mL), complete medium 10%, O serum (10 mL) was added and filtered using minisart (0.45 µm followed by 0.2 µm).

Preparation of *P. falciparum* culture
Frozen *P. falciparum* 3D7 strain chloroquine sensitive was warmed at 37^{o}C. The parasites containing solution were transferred in to centrifuge tube (15 mL) and 12% NaCl solution was added dropwise. After 5 minutes, 9 times volume of 1.6% NaCl was added dropwise and the mixture was then centrifuged at 2000 rpm for 5 minutes. The packed cells for culture were prepared in 20% of complete medium in the tissue culture flask.

Parasitemia determination by optical microscopy
Culture flask was incubated at 37^{o}C in 5% CO_2, 95% O_2 incubator and the medium was changed everyday. After two days, parasitemia were counted in thin smear plate. If the viability is on the range of 2-5%, RBC (50-100 µL) was added and re-incubated (the parasite can be sub-cultured if the parasitemia about 10%). The culture was relocated into tube and was centrifuged at 2000 rpm for 5 minutes. To the packed cells, were added with complete medium (10%, 5 mL) was added. The cells were then divided into 2 flasks and complete medium (3 times of volume of parasites culture) and RBC were added and incubated until the parasitemia 10% was achieved.

Thin smear was obtained by spreading erythrocytes on to object glass. The thin smear was fixed with methanol, stained by 20% of Giemsa and left for 20 minutes. The slides were then washed with water and dried. Parasitemia were counted under optical microscopy and the % of parasitemia was determined using formula:

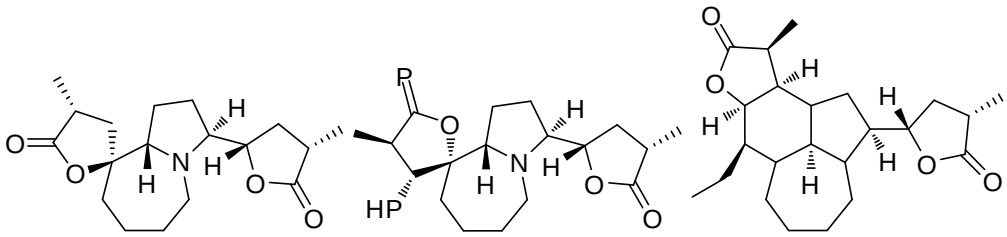
$$\% \, Parasitemia = \frac{\sum infected \; RBCi}{1000 \; RBC} x \; 100\%$$

Parasitemia determination by flow cytometry
Mapping concentrations on tissue culture well 96 and put 20 µL of ≥10% parasitemia cells into tissue culture well 96. The sample of alkaloids, croomine, *epi*-croomine, tuberostemonine and crude were added 280 µL of each and replication until their final concentrations are 0.01; 0.1; 1.0; 10 ppm, respectively. The negative control was contains parasites and complete medium. The cultures were incubated at 37^{o}C in the 5% $CO_2$and 95% O_2 incubator for 48 hours. The 20 µL of erythrocytes was took to glass for thin smear and the balance for flow cytromery analysis. All the concentrations are performed double replication. Each of wells from in vitro cultures were separately relocated into tubes and centrifuged at 450 rpm for 5 minutes, the pellet cells were washes with 1 mL of PBS. The tubes were then centrifuged at 450 rpm for 5 minutes and fixation of the cells were conducted in glutaraldehyde (0.25%) containing PBS (0.5 mL). Each of cells was incubated at room temperature for 20 minutes and centrifuged at 450 rpm for 5 minutes. The cells were washed with 1 mL of PBS in twice, then suspension of cells in 0.5 of PBS containing 0.01% of saponin and incubated at room temperature for 5 minutes. The cells were washed again twice

with 1 mL of cold PBS. To the cells, PBS (200 µL) and RNAse (1 ppm, 40 µL) were added and cells were then incubated at 37^{o} C for 60 minutes. Each of cells was washed twice with cold PBS (1 mL) and centrifuged at 450 rpm for 5 minutes. The cells were stained with propidium iodide (500 µL) and incubated at 37^{o} C temperature for 60-120 minutes on dark room. All the tubes were analyzed by FCM BD FACS-Calibur on λ = 488/460 (ex/em) Cell Quest Pro-red Channel FL3 mode.

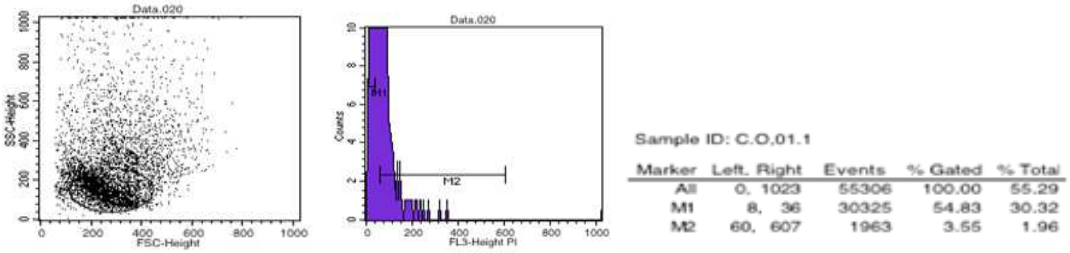
RESULTS AND DISCUSSION

The alkaloids croomine **1**, *epi*-croomine **2** and tuberostemonine **3** (Figure 1) were previously isolated from *S. tuberosa*; compound **1** and **2** were reported to competitively inhibit DHFR at 10 ppm. The computational studies revealed the lactone ring of the alkaloid molecule contributed into providing hydrogen bonding at Ala amino acid residue on the active side [20].



1 **2** **3**

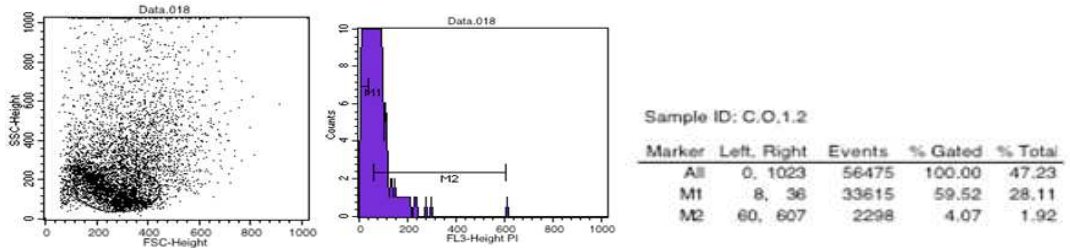
Figure 1. Anti-malarial active alkaloid constituents of *S. tuberosa* Lour from Indonesia



Sample ID: C.O.01.1

Marker	Left, Right	Events	% Gated	% Total
All	0, 1023	55306	100.00	55.29
M1	8, 36	30325	54.83	30.32
M2	60, 607	1963	3.55	1.96

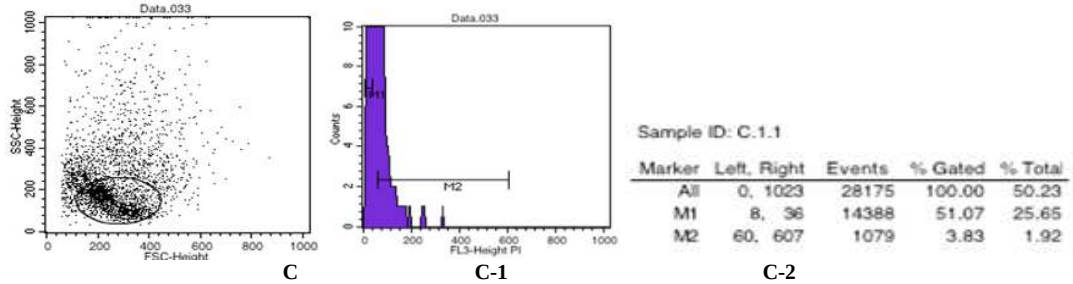
A **A-1** **A-2**



Sample ID: C.O.1.2

Marker	Left, Right	Events	% Gated	% Total
All	0, 1023	56475	100.00	47.23
M1	8, 36	33615	59.52	28.11
M2	60, 607	2298	4.07	1.92

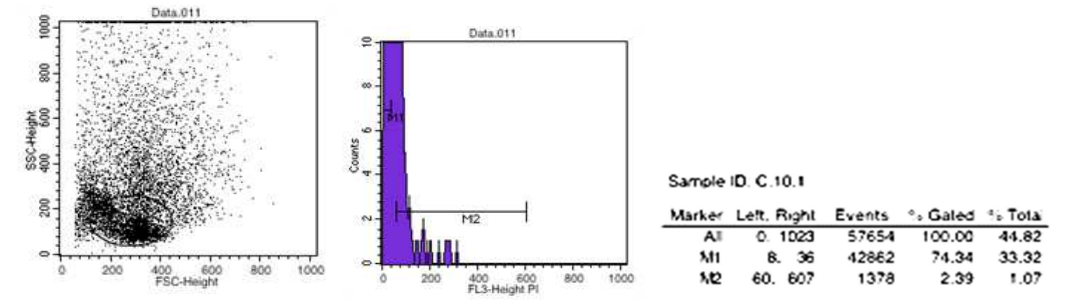
B **B-1** **B-2**



Sample ID: C.1.1

Marker	Left, Right	Events	% Gated	% Total
All	0, 1023	28175	100.00	50.23
M1	8, 36	14388	51.07	25.65
M2	60, 607	1079	3.83	1.92

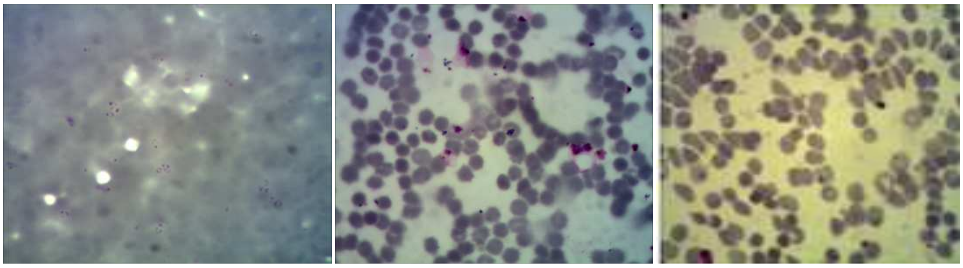
C **C-1** **C-2**



Sample ID: C.10.1

Marker	Left, Right	Events	% Gated	% Total
All	0, 1023	57654	100.00	44.82
M1	8, 36	42862	74.34	33.32
M2	60, 607	1378	2.39	1.07

D **D-1** **D-2**



E **F** **G**

Figure2. FCM analysis of parasitemia from *P. falciparum* parasitized erythrocyte of 3D7 strain stained with propidium iodide for 48 hours. On the left region, Figures (A, B, C and D) represent a two dimensional plot in light scatter of parasites population in treatment with croomine at concentration 0.01; 0.1; 1.0 and 10 ppm, respectively. On the middle region, Figures (A-1, B-1, C-1 and D-1) represent histogram markers of events. On the right region, Figure (A-2, B-2, C-2 and D-2) represent of histogram statistical percentages of the negative and positive, compared by event counts with the gated event. Giemsa-stained thin smears (E, F, G)

The parasitemia determination using Giemsa smear is simple, cheap, sensitive and accurate but high skill and time is required. In the other hand, the FCM method is quick, specific but more expensive. Here both methods were used and the data were visualized. Propidium iodide is able to intercalate with nucleic acids and acts as fluorescence molecules which is detected as single fluorescent signal in FCM [22]. This concept is similar with the use of trypan blue staining agent in OM. The damaged cells (fragments) were identified automatically on population of forward-scattered (FSC) as axis and side-scattered light (SSC) as ordinate (Figure 2, A, B, C and D). FSC is diffracted light for detecting particles size and SSC is refracted and reflected light. On the middle histogram (Figure 2, A-1, B-1, C-1 and D-1), marker M1 is a negative peak and indicate non fluorescence event commonly uninfected erythrocyte, also dead parasite inside [15]. Marker M2 is positive event, corresponded to fluorescence peak. Parasitemia value represent (Figure 2, A-2, B-2, C-3 and D-4) are % gated of M2, include 3.66; 4.07; 3.82; 2.39. The results of determination of parasitemia of alkaloids to the *P. falciparum* in vitro tissue culture were determined by OM and FCM (Table 1).

Table 1. The mean of parasitemia % of alkaloid by flow cytometry and optical microscopy

Alkaloid	Concentration (ppm)	FC	OM	p
croomine	10	4.5100 ± 0.18385	4.0000 ± 0.50912	NS
	1.0	4.1050 ± 0.21920	4.7300 ± 0.55154	NS
	0.1	6.1250 ± 0.09192	4.2100 ± 0.38184	NS
	0.01	5.0300 ± 0.02828	4.5000 ± 1.24451	NS
epi-croomine	10	3.9350 ± 0.07778	4.8650 ± 0.31820	NS
	1.0	5.6550 ± 0.24749	6.3650 ± 0.81317	NS
	0.1	5.2250 ± 0.06364	7.7650 ± 0.43134	NS
	0.01	5.5250 ± 0.33234	6.6300 ± 0.48083	NS
tuberostemonine	10	4.7600 ± 0.45255	4.0100 ± 0.07071	NS
	1.0	5.3700 ± 0.18385	5.5300 ± 0.70711	NS
	0.1	5.3600 ± 0.02828	5.4350 ± 1.23744	NS
	0.01	5.1650 ± 0.14849	2.6550 ± 3.74059	NS
crude	10	2.2500 ± 0.19799	2.1950 ± 0.12021	NS
	1.0	3.5950 ± 0.33234	1.9950 ± 0.71418	NS
	0.1	4.2150 ± 0.20506	1.2050 ± 0.09192	<0,05 (S)
	0.01	3.6150 ± 0.09192	1.3050 ± 0.75660	NS

In generall, determination of parasitemia of the all concentration of alkaloids from *S. tuberosa* Lour using both OM and FCM method showed insignificance differences statistically, except crude alkaloid at 0.1 ppm concentration. This clearly indicated that the cost effective traditional OM method has good sensitivity and reliable.

CONCLUSION

Optical microscopy (OM) and flow cytometry (FCM) methods have similar ability for counting parasitemia. Despite some limitations, OM method is cheaper and become a gold standard method due to its additional capability to distinguish species and stage development of *Plasmodium*. These have valued the OM method availability in developing country with endemic malaria.

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REFERENCES

[1] World Health Organization. **2012,** World malaria report
[2] C.Wongsrichanalai, M. J. Barcus, S.Muth, A.Sutamihardja, W. H. Wernsdorfer, *Am. J. Trop. Med. Hyg.*, **2007**, 77, Suppl 6, 119–127
[3] H.M. Shapiro, F. Mandy, International Society for Analytical Cytology, Cytometry Part A 71A, **2007**, 643-645
[4] W.Roobsoong, S.P.Maher, N. Rachaphaew, S.J. Barnes, K.C. Williamson, J.Sattabongkot, J.H. Adams, *Malar. J.*, **2014**, 13, 55
[5] J.D. Hare, D.W. Bahler, J. Donald,*J. Histochem.Cytochem.*,**1986**, 34, 215-220
[6] G. Jun, J. S. Lee, Y. J. Jung, J. W. Park**,** *J. Korean Med. Sci*., **2012**, 27, 1137-1142
[7] S. Karl, R.P.M. Wong, T.G.St Pierre, T.M.E. Davis, *Malar. J.*, **2009**, 8, 294
[8] M.L. Varela, R.Razakandrainibe, D.Aldebert, J.C.Barale, R.Jambou, *Malar. J.*,**2014**, 13, 110
[9] M. Rebelo, C. Sousa, H. M. Shapiro, M. M. Mota, M. P. Grobusch, T. Hanscheid, *PLoSONE*, **2013,** 8, 61606
[10] B.T. Grimberg, *J. Immunol. Meth.*, **2011**, 367, 1–16
[11] C.E. Contreras, M. A. Rivas, J.Domínguez, J.Charris, M. Palacios, N.E.Bianco, I. Blanca, *Mem. Inst. Oswaldo Cruz.*, **2004**, 99,179-184
[12]J.J. Campo, J.J. Aponte, A.J. Nhabomba, J.Sacarlal, I. A. Barturen, M.B.J. Díaz, P.L. Alonso, C.Doban, *J.Clin.Microbiol.*, **2011**, 968–974
[13] A. Saito-Ito, Y. Akai, S. He, M. Kimura, M. Kawabata, *Parasitol. Int.*, **2001**, 50, 249
[14]D.C. Schuck, R.Y. Ribeiro, A.A. Nery, H. Ulrich, C.R.S. Garcia, *CytometryA*., **2011**, 71, 959-964
[15]S. Chevalley, A. Coste, A. Lopez, B. Pipy, A. Valentin, *Malar. J.*, **2010**, 9, 49
[16]M.B.J.Dıaz, J. Rullas, T. Mulet, L. Fernandez, C. Bravo, D.G. Viola, I.A. Barturen, *CytometryA*, **2005**, 67, 27–36
[17] Q. Li, L.Gerena, L.Xie, J. Zhang, D. Kyle, W. Milhous, *CytometryA*, **2007**, 71, 297–307
[18] R.W. Tiendrebeogo, B. Adu, S. K. Singh, D. Dodoo, M.H Dziegiel, B. Mordmüller, I. Nébié, S.B. Sirima, M. Christiansen, M. Theisen, *Malar. J.*, **2014**, 13, 412
[19] A.S. Nugraha, P.A. Keller, *Nat. Prod. Comm.*, **2011**, 6, 1953-1966
[20] P. Pudjiastuti, S. Sumarsih, H. Arwati, I. Amalina, MZ. Fanani, EP. Utomo, , LE. Fitri, AS. Nugraha, W. Lie, SG. Pyne, *Journal of Pharmaceutical and Chemistry Research*, **2014**, 6(6): 544-548
[21] P. Pudjiastuti, S.G. Pyne, Sugiyanto, Wilford Lie, *Phytochem.Lett.*, **2012**, 5, 358-360
[22] V. Wongchotigul, N. Suwanna, S. Krudsood, D. Chindanond, S. Kano, N. Hanaoka, Y. Akai, Y. Maekawa, S. Nakayama, S. Kojima, S. Looareesuwan, *Southeast Asian J. Trop. Med. Public Health*, **2004**, 35, 552-559