Research Journal of Pharmaceutical, Biological and Chemical Sciences

(An International Research Online Journal)

ISSN:0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

ISSN:0975-8585

Invitation for Innovative Research Papers, Reviews, Mini Reviews and Short Communication

Editorial Board

Research Journal of Pharmaceutical, Biological and Chemical Sciences

ISSN:0975-8585

Conference Special Supplementary Issue.

RES J PHARM BIOL CHEM SCI
8(1S), 2017 (January - February) Supplementary Issue

The Tittle:
Second International Seminar on Pharmaceutical Sciences and Technology 2016 (2nd ISPST2016) November 24th and 25th 2016.

Venue:
Bale Sawala, Rektorat Universitas Padjadjaran, Jl Raya Bandung Sumedang km 21.5 Jatinangor, West Java, Indonesia

Research Journal of Pharmaceutical, Biological and Chemical Sciences

discontinued in Scopus as of 2016

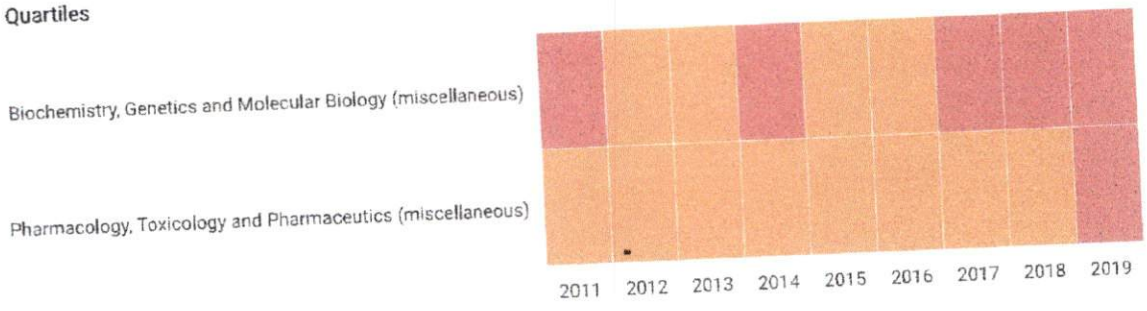
Country	India -
Subject Area and Category	Biochemistry, Genetics and Molecular Biology Biochemistry, Genetics and Molecular Biology (miscellaneous) Pharmacology, Toxicology and Pharmaceutics Pharmacology, Toxicology and Pharmaceutics (miscellaneous)
Publisher	RJPBCS
Publication type	Journals
ISSN	09758585
Coverage	2010-2016
Scope	Information not localized

27

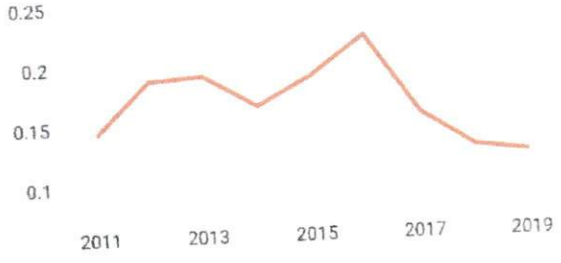
H Index

Quality Supplements at iHerb®
iHerb

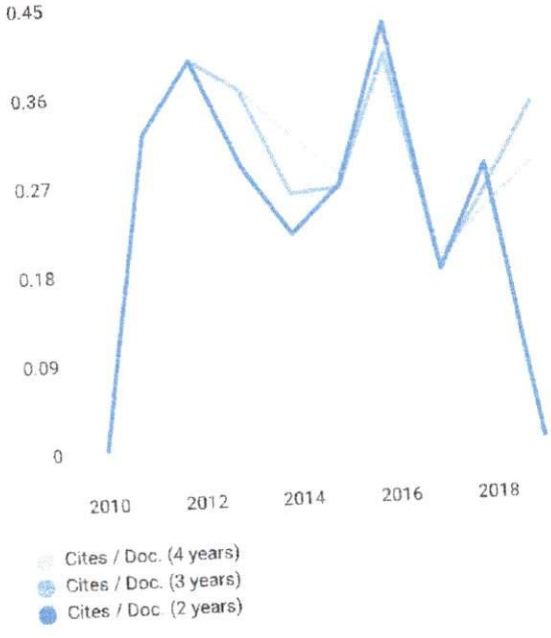
Quartiles



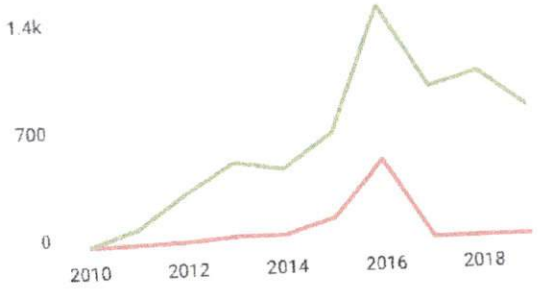
SJR



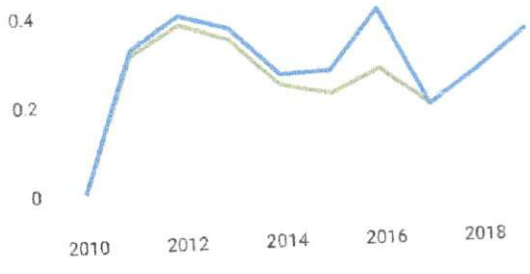
Citations per document



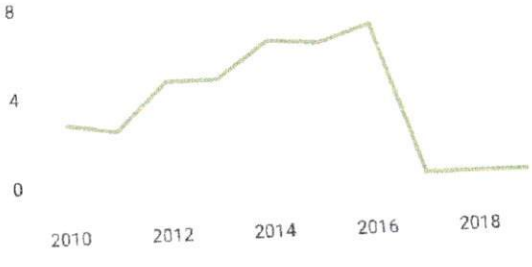
Total Cites Self-Cites



External Cites per Doc Cites per Doc



% International Collaboration

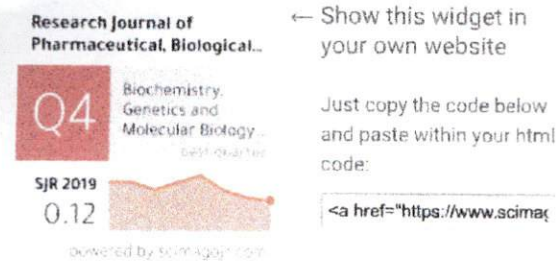


Citable documents Non-citable documents

Cited documents Uncited documents

6k

6k



Metrics based on Scopus® data as of April 2020

Ekata kumari 3 months ago

When it is in ugc list how to prove it's in ugc list

reply

Melanie Ortiz 3 months ago

Dear Ekata,
Thank you for contacting us.
SJR is a portal with scientometric indicators of journals indexed in Elsevier/Scopus. Unfortunately, we cannot help you with your request referring to the index status. We suggest you consult Scopus database (see the current status of the journal) or the mentioned database for further information. You can also check that information in the journal's website or contact directly with the editorial staff.
Best Regards, SCImago Team

Dr Nada Ahmedzeki 1 year ago

Dear,
The status of the journal says that it was cancelled, I want to know if the year 2016 is included in SJR or not. This information is quite important for a paper published in Nov.Dec. 2016.

reply

(19)

ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Antimalarial Activity and Survival Time of *Andrographis paniculata* Fraction (AS202-01) on *Plasmodium berghei* Infected Mice.

Aty Widyawaruyanti[1,3*], Deri Astrianto[2], Hilkatul Ilmi[3], Lidya Tumewu[3], Dwi Setyawan[1], Endang Widiastuti[5], Lilis Dachliyati[5], Indah S Tantular[3,4], and Achmad Fuad Hafid[1,3].

[1]Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Dharmawangsa Dalam, Surabaya-60286
[2]Undergraduate student of Faculty of Pharmacy, Universitas Airlangga, Dharmawangsa Dalam, Surabaya-60286
[3]Institute of Tropical Disease, Universitas Airlangga, C Campus Universitas Airlangga, Mulyorejo, Surabaya-60115
[4]Department of Parasitology, Faculty of Medicine, Universitas Airlangga, Mayjen. Prof. Dr. Moestopo 47, Surabaya-60131
[5]PT. Kimia Farma (Persero) Tbk., Veteran No.9, Jakarta-10110

ABSTRACT

The rapid spread of resistance encourages the search for new active compounds. *Andrographis paniculata* commonly known as sambiloto was empirically used as traditional medicine in Indonesia to cure malaria. This study aims to determine antimalarial activity and survival time of *A. paniculata* fraction from 70% ethanolic extract (namely AS202-01) on *Plasmodium berghei* infected mice. Antimalarial in vivo study was conducted based on Peter's 4-days suppressive test. Thirty healthy Balb/c male mice, divided into 6 groups (n = 5), were infected intravenously with $1x10^6$ parasitized erythrocytes of *P. berghei*. Infected mice were treated at a dose of 6.25, 12.5, 25 and 50 mg/kg BW, twice a day for four days, the untreated group of mice received CMC-Na 0.5% and the control group treated with chloroquine (10 mg/kg BW). Thin blood smears giemsa staining were made every day for seven days and observed under microscope to count parasitemia and calculate inhibition of parasite's growth. Probit analysis was conducted to determine effective dose (ED_{50}) value. Observation of antimalarial effect of AS202-01 was continued until 14 days to evaluate survival time of infected mice. The results showed that AS202-01 was inhibited *P. berghei* growth with ED_{50} value of 6.75 mg/kg BW. It was classified as a highly active antimalarial substance. The AS202-01 was also significantly able to increase survival time of infected mice compared to untreated group.

Keywords: *A. paniculata* fraction (AS202-01), antimalarial, survival time, *Plasmodium berghei*

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Malaria is one of the most important infectious diseases in the word. This disease is caused by Plasmodium species and transmitted by the bite of female Anopheles mosquito [1]. Estimated 214 million people infected malaria in the word. Despite the decrease of malaria infection about 18% from 2000 to 2015, but a new problem is antimalarial drug resistant Plasmodium parasites and the emergence of insecticide resistant Anopheles [2]. Resistant not only the spread of malaria to new areas but also its reemergence in areas where it had previously been eradicated [3]. Therefore, the search for new antimalarial, either synthetic or natural, is important for killing of malaria parasites [4].

The use of medicinal plant extract for treatment of malaria has a long and successful tradition [5]. For example, quinine was isolated from *Chinchona* species (Rubiaceae) and artemisinin from *Artemisia annua* [6]. Diterpenoids, flavonoids, saponin, polyphenols, alkaloids, kaempferol, and acetogenin from plants were known to have antimalarial activity [7, 8]. This explains why a lot of current researches focus on natural and plant-derived products as they can be sourced easily, can be locally available, and can be selected on the basis of their pharmacological use [9, 10].

Andrographis paniculata commonly known as sambiloto was empirically used as traditional medicine in Indonesia to treat people suffering from malaria. It also possesses antimicrobial effect, antiimflamantory, antioxidant, antidiabetic, antiHIV, antifungal, hepatoprotector, antifertility and anti cancer [11, 12]. The major constituents of *A. paniculata* is andrographolide, a diterpene lactone compound, which has antimalarial activity both in vitro and in vivo [13]. Andrographolide inhibited *P. berghei* growth with ED_{50} value of 3.82 mg/kgBW [14]. The ethyl acetate fractions of *A. paniculata* from 70% ethanolic extract (AS202-01) is an andrographolide rich fraction. Previous study reported that andrographolide content of AS202-01 was higher among other extracts and fraction [15]. This study aims to determine antimalarial activity and survival time of AS202-01 on *Plasmodium bergei* infected mice.

MATERIALS AND METHODS

Plant materials:

A. paniculata dried powder with batch number PP01J1125027 was obtained from PT. Kimia Farma (Persero) Tbk, Bandung, Indonesia on February 2015

Experimental animals:

Male mice BALB/C strain were obtained from LPPT-Universitas Gajah Mada, Yogyakarta. They were weighting between 20-30 g and maintained on standard animal pellets and water ad libitum at Animal Laboratory of Institute of Tropical Disease, Universitas Airlangga. Permission and approval for animal studies were obtained from Faculty of Veterinary Medicine, Universitas Airlangga No:489-KE/2015.

Rodent malaria parasite

Plasmodium berghei ANKA strain was originally obtained from Eijkman Institute for Molecular Biology, Jakarta. The parasite has been maintained at Institute of Tropical Disease, Universitas Airlangga by a combination of passage in male BALB/C mice and cryoscopic storage.

Methods:

Procedure 1: Preparation of *A. Paniculata* fraction (AS202-01)

A. paniculata dried powder was extracted by maceration method using ethanol 70% as a solvent. Liquid ethanol extract then evaporated using rotary evaporator. The concentrated extract then further separation by liquid-liquid fractionation using ethyl acetate and water to obtain ethyl acetate fraction namely AS202-01.

INTRODUCTION

Malaria is one of the most important infectious diseases in the world. This disease is caused by Plasmodium species and transmitted by the bite of female Anopheles mosquito [1]. Estimated 214 million confirmed infected malaria worldwide. The reported malaria infection about 198 from 2000 to 2015 [2]. New medicine is urgently required due to the parasite's resistance to drugs and the emergence of insecticide resistance mosquitoes [3]. Resistant not only the vector or malaria to new drugs but also the emergence in cross-resistance to previously used or introduced [4]. Therefore, the search for new antimalarial either synthetic or natural is important for the development of malaria eradication [4].

The use of medicinal plant extract for treatment of malaria has a long and successful tradition [5]. For example, quinine was isolated from Cinchona species (Rubiaceae) and artemisinin from Artemisia annua [6]. Diterpenoids, flavonoids, saponin, polyphenolic, alkaloid, terpenoid, and sesquiterpene lactones were known to have antimalarial activity [7, 8]. This explains why a lot of current researches focus on natural and plant derived products as they can be easily isolated, relatively available, and can be selected on the basis of their pharmacological activity [9].

Andrographis paniculata commonly known as hempedu bumi was empirically used as traditional medicine in Indonesia to treat people suffering from malaria. It has possesses antimicrobial effect, antioxidant, antioxidant, antidiabetic, antiviral, antifungal, hepatoprotective, antiferility and anti cancer [11, 12]. The major constituents of A. paniculata is andrographolide [13], a diterpene lactone, mainly soluble in water and its antimalarial activity both in vitro and in vivo [13]. Andrographolide inhibited P. berghei growth with IC50 value of 3.8 mg/kgBW [14]. The ethyl acetate fractions of A. paniculata from 70% ethanolic extract (AS202-01) is an andrographolide rich fraction. Previous study reported that andrographolide content of AS202-01 was higher among other extracts and fraction [15]. This study aims to determine antimalarial activity and survival time of AS202-01 on Plasmodium berghei infected mice.

MATERIALS AND METHODS

Plant materials:

A. paniculata dried powder with batch number P2011-12-017 was obtained from PT. Konimex Farma (Formerly Tbk), Sragung, Indonesia on February 2015.

Experimental animals:

Male mice BALB/C strain were obtained from LPPT Universitas Gadjah Mada, Yogyakarta. They were weighing between 20-30 g and maintained on standard animal pellets and water ad libitum at Animal Laboratory of Institute of Tropical Disease, Airlangga University. Permission and approval for animal studies were obtained from Faculty of Veterinary Medicine, Universitas Airlangga No. 489-KE/2015.

Rodent malaria parasite

Plasmodium berghei ANKA strain was originally obtained from Eijkman Institute for Molecular Biology, Jakarta. The parasite has been maintained at Institute of Tropical Disease, Universitas Airlangga by a combination of passage in male BALB/C mice and cryopreservation in vapor.

Methods:

Procedure 1: Preparation of A. Paniculata fraction (AS202-01)

A. paniculata dried powder was extracted by maceration method using ethanol 70% as a solvent. Liquid ethanol extract then evaporated using rotary evaporator. The concentrated extract then further separation by liquid-liquid fractionation using ethyl acetate and water, to obtain ethyl acetate fraction namely AS202-01.

Procedure 2: In vivo antimalarial activity test

In vivo antimalarial activity was performed based on Peter's test (The 4-days suppressive test) [16]. AS202-01 was tested using 30 mice which divided to 6 groups (n=5): untreated group (CMC-Na 0.5%), control group (chloroquin), AS202-01 dose of 6.25 mg/kg BW, AS202-01 dose of 12.5 mg/kg BW, AS202-01 dose of 25 mg/kg BW, AS202-01 dose of 50 mg/kg BW.

Each mice was infected intraperitoneally with 0.2 ml *P. berghei* (1 x 10^6). Infected mice were treated at a dose of 6.25, 12.5, 25 and 50 mg/kg BW, twice a day for four days. The untreated group of mice received CMC-Na 0.5% and control group (chloroquin at dose 10 mg/kg BW). Thin blood smears were made every day for 5 days (day-0 until day-4) and stained using 10% giemsa dye. Thin blood smear were observed under microscope to count the percentage of parasitemia and calculate inhibition of parasite's growth.

Procedure 3: Determine of mean survival time (MST)

Determination of MST was performed based on Somsak et al, 2016 [4]. The survival time of *P. berghei* infected mice was observed during 14 days (day-0 until day-13).

Data Analysis

In vivo antimalarial activity test

Percentage of parasitemia and percentage of inhibition growth of *P. berghei* were calculated using the formula below.

Percentage of parasitemia:

$$\frac{Xe}{Xk} X\ 100\%$$

Percentage of inhibition:

$$100\% - (\frac{Xe}{Xk} X\ 100\%)$$

Xe: % parasitemia growth of experimental group

Xk: % parasitemia growth of untreated group

Inhibition pecentage was analyzed using Probit analysis SPSS to determine Effective Dose (ED_{50}) value.

Determine of mean survival time (MST)

The mean survival time (MST) was calculated using the formula below.

$$MST = \frac{\text{Sum of survival time of all mice in a group}}{\text{Total number of mice in that group}}$$

RESULTS AND DISCUSSION

Antimalarial Activity

Andrographis paniculata belongs to the family Acanthaceae that has been used as traditional medicine in Indonesia to cure malaria. Many reports are also available on the antimalarial activity of *A. paniculata.* Mishra (1992) was reported that crude ethanol extract and fractions of *A. Paniculata* inhibited *P.*

berghei growth. Methanol, chloroform, and petroluem ether extract of *A. paniculata* inhibited *P. falciparum* growth on gametocytes and schizont stage [17,18].

The present study assessed the antimalarial activity of AS202-01, *A. Paniculata* fraction obtained from 70% ethanol extract of *A. Paniculata*. The results showed than AS202-01 inhibited parasite (*P. berghei*) growth. The average of parasitemia percentage of infected mice which treated by AS202-01 on first-day observation until fifth day (D0-D4) tended to increase, but it was still lower than untreated group, this is showed that AS202-01 inhibited the growth of *P. berghei* (ANKA strain) in mice. The results are summarized in table 1.

Table 1. Activity of AS202-01 on *P. bergehi* infected mice

Group	Dose (mg/kgBW)	% Parasitemia					% average Inhibition parasite's growth
		H0	H1	H2	H3	H4	
Negative Control		0.21±0.04	2.16±1.06	5.14±1.07	7.90±1.33	10.46±2.20	-
Chloroquin	10	0.32±0.15	0.31±0.11	0.13±0.05	0.20±0.15	0.23±0.24	99.47±0.79
AS202-01	6.25	0.19±0.06	1.05±0.50	2.09±0.70	3.29±0.23	4.86±0.24	48.33±2.25
	12.5	0.30±0.11	1.26±0.40	2.19±0.61	3.07±0.44	4.24±0.26	58.46±1.27
	25	0.19±0.10	0.91±0.37	1.31±0.47	2.28±0.78	3.33±0.10	64.81±1.27
	50	0.27±0.09	0.73±0.46	1.16±0.31	1.82±0.55	2.86±0.15	70.16±0.78

AS202-01 : *A. paniculata* fraction from
H0-H4 : Day of observation

The data on table 1 showed that inhibition parasite's growth followed a dose dependent manner. The data of inhibition parasite growth was analyzed using log-probit to determine Effective Dose (ED_{50}) value. The result analysis showed that AS202-01 has ED_{50} value of 6.75 mg/kg BW, it was highly active as an antimalarial substance based on criteria Munoz et al (2000) [19]. The period of activity of andrographolide was found evidently on the ring stage of the parasite. The point of action of this compound in the parasite life cycle corresponds with the protein and nucleic acid synthesis [13]. *A. paniculata* extract and andrographolide includes inhibition of the nuclear transcription factor-kappa B (NF-κB), making it a therapeutic target for the treatment of cancer and autoimmune diseases [20]. The role of NF-κB is also important in malaria as mentioned in a recent study [21]. Plasmodium infected erythrocytes have shown to induce NF-B (kappa) regulated inflammatory pathways in human cerebral endothelium. NF-κB is activated and translocate into nucleus, where it binds to the DNA regulatory site to regulate specific gene expression, especially cell signaling for parasite growth and development. Therefore, the inhibition of transcription factor of *A. paniculata* extract against *P. berghei* ANKA might be a critical process to inhibit blood stage propagation of parasites in vivo [12, 20].

This study results showed that chloroquine had antimalarial activity higher than AS202-01, its inhibition parasite's growth with the value of 99.47%±0.79 at a dose of 10 mg/kg BW. Meanwhile, AS202-01 at the highest dose (50 mg/kg BW) inhibited parasite's growth with the value of 70.16%±0.78. The difference of these activites might be due to the life time of Andrographolide was 2-7 hours, and rapidly elimination within 3-4 hours after administration[22], and the life time of Chloroquine was 150 hour or 6 day on healthy people.

Survival Time

The result showed AS202-01 had higher survival time than negative control. AS202-01 at all doses was capable of significantly increasing the survival time. Survival time was showed dose dependent manner. The higher of AS202-01 dose, then survival time was higher as well. The results are summarized in table 2.

The survival time longer than 12 days by the test compound is regarded as active [23]. AS202-01 at a dose of 12.5 until 50 mg/kg BW was performed survival time more than 12 days. Moreover, survival time which observed was almost as chloroquine for AS202-01 at the highest dose (50 mg/kg BW).

ISSN: 0975-8585

The data on table 2 also showed that the higher of inhibition parasite's growt, then survival time was higher as well. It was posible related to the immune system of infected mice. *A. paniculata* was reported as immunosuppresif agent [24]. Based on the facts, AS202-01 might plays a role in immune system which inhibit parasite's growth and then the condition was assosiated with increased survival time.

Table 2. Survival time of AS202-01

Group	Dose (mg/kgBW)	% average Inhibition parasite's growth	Survival time (Day)
Negative control		-	9.6±2.7
Chloroquin	10	99.47±0.79	14±0
AS202-01	6.25	48.33±2.25	11.8±3.03
	12.5	58.46±1.27	12.6±2.19
	25	64.81±1.27	13.2±1.79
	50	70.16±0.78	13.8±0.45

AS202-01: *A. paniculata* fraction

CONCLUSIONS

AS202-01 was active as an antimalarial on *Plasmodium berghei* infected mice with ED_{50} value of 6.75 mg/kg BW and classified as a highly active antimalarial substance. The AS202-01 was significantly able to increase survival time compared to negative control.

ACKNOWLEDGEMENTS

The authors acknowledge grant support from Indonesian Directorate General of Higher Education DIPA BOPTN 2016, contract no 951/UN3.14/2016.

REFERENCES

[1] WHO.2009. Guidelines for the treatment of malaria. Second edition. Availabl at: http://whqlibdoc.who.int/publications/2010/9789241547925_enf.pdf. Accessed: 1 November 2016.
[2] WHO. 2015. World Malaria Report 2015. WHO Geneva.
[3] Barik T. 2015. Antimalarial drug: from its development to deface, Current Drug Discovery Technologies, vol. 12, no. 4, pp. 225–228.
[4] Somsak V., Polwiang N., Chachiyo S. 2016. In Vivo Antimalarial Activity of *Annona muricata* Leaf Extract in Mice Infected with *Plasmodium berghei*. Journal of Pathogens, 10: 1-5.
[5] Kaur K., Jain M., Kaur T., and Jain R. 2009. Antimalarials from nature," Bioorganic & Medicinal Chemistry, vol. 17, no. 9, pp.3229–3256.
[6] Mutabingwa T.K. 2005. Artemisinin-based combination therapies (ACTs) best hope for malaria treatment but inaccessible to the needy. Acta Trop;95:305-15.
[7] Ginsburg H and Atamna H. 1994. The redox status of malaria infected erythrocytes: an overview with an emphasis on unresolved problems, Parasite, vol. 1, no. 1, pp. 5–13.
[8] Barliana M.I., Suradji E.W., Abdulah R. 2014. Antiplasmodial properties of kaempferol-3-O-rhamnoside isolated from the leaves of *Schima wallichii* against chloroquine-resistant *Plasmodium falciparum*, Biomedical Reports, vol. 2, no. 4, pp. 579-583.
[9] Negi A.S., Gupta A, and Hamid A.A. 2014. Combating malaria with plant molecules: a brief update, Current Medicinal Chemistry,vol. 21, no. 4, pp. 458–500.
[10] Mojab F. 2012. Antimalarial natural products: a review, Avicenna Journal of Phytomedicine, no. 2, pp. 52–62.
[11] Niranjan, Abhishek, Tewari S.K., Lehri A. 2010. Biological Activities of Kalmegh (*Andrographis paniculata* Nees) and Its Active Principle-A review. Indian Journal of Natural Products and Resources Vol 1(2): 125 – 135
[12] Mishra K, Dash A.P., Swain B.K., Dey N. 2009. Antimalarial activities of *Andrographis paniculata* and *Hedyotis corymbosa* extracts and their combination with curcumin. Malaria Journal; 8 (26), 1-9.

...ble 2 also shows that the blend of both molecules showed a good mean survival time was ...well. It was possible related to the immune system of the host since A. pernyi ...ted in ...mmunocompetent agent [29]. Based on the facts A5202-01 might play a role in immune system which might possible growth and then the condition was regulated with time to survival time.

Table 2. Survival time of A5202-01

Group	Dose (mg/kgBW)	% average inhibition parasite's growth	Survival time (Day)
The above control			6 ± 2 d
Chloroquin	20	99.17±0.29	14±0
A5202-01	0.2	40.33±2.27	12.67±1.05
	1.4	50.46±2.27	12±2.19
	28	64.81±1.27	13.2±1.79
	56	70.19±0.28	12.8±0.45

A5202-01: A. pannellata fraction

CONCLUSIONS

A5202-01 was active as an antimalarial on Plasmodium berghei infected mice with ED50 value of 1.75 mg/kg BW and classified as a highly active antimalarial substance. The A5202-01 was significantly able to increase the survival time compared to negative control.

ACKNOWLEDGEMENTS

The authors acknowledge grant support from Indonesian Directorate General of Higher Education DIPA BOPTN 2015, contract no 951/UN9.3/LT/2015.

REFERENCES

[1] WHO.2009. Guidelines for the treatment of malaria Second edition. Available at: http://whqlibdoc.who.int/publications/2010/9789241547925_eng.pdf. Accessed: 3 November 2015.

[2] WHO. 2015. World Malaria Report 2015. WHO Geneva.

[3] Barik T. 2015. Antimalarial drug from its development to delivery. Current Drug Discovery Technologies, vol.12 no. 4 pp 222-226.

[4] Sonnak V., Polwiang N., Chaichana S. 2014. In Vivo Antimalarial Activity of Annona muricata Leaf Extract in Mice Infected with Plasmodium berghei. Journal Pathogens, 39: 1-5.

[5] Nour K., Jain M., Kaur T., and Jain R. 2013. Antimalarials from nature. Bioorganic & Medicinal Chemistry vol.17 no. 9 pp 3229–3256

[6] Nurtabinova T.K. 2008. Artemisinin-based combination therapies (ACTs) best hope for malaria treatment but inaccessible to the needy. Acta Trop;95:305–15.

[7] Ginsburg H and Atamna H. 1994. The redox role of malaria infected erythrocytes: an overview with an emphasis on unresolved problems. Parasite, vol 1 no 1 pp 5-13

[8] Barliana M.I., Surasdi E.M., Abdullah F. 2014. Antiplasmodial Alkaloids Isolated from the Leaves of Carica papaya Cultivated Against Chloroquine Resistant Plasmodium falciparum, Biomedical Reports, vol. 2 no 4, pp 581-583.

[9] Tsegi A.S., Gupta D., and Han A.A. 2014. Combating malaria with plant molecules: a brief update, Current Medicinal Chemistry vol. 21 no 4 pp 458-500

[10] Molyb K. 2012. Antiplasmodial natural products: a review, an Indian journal of Phytomedicine, no. 1 pp. 52-62

[11] Niranjan, Abhishek, Tevtani Suri, Lehri A. 2010. Biological Activities of Kalmegh (Andrographis paniculata Nees) and Its Active Principle-A review, Indian Journal of Natural Products and Resources Vol 1(2): 127 – 135

[12] Mishra K. Dash A.P., Swain B.K., Dey N. 2009. Antimalarial activities of Andrographis paniculata and Hedyotis corymbosa extracts and their combination with curcumin. Malaria Journal, 8 (26): 1-9

[13] Mishra K, Dash A.P., Swain B.K., Dey N. 2011. Andrographolide: A Novel Antimalarial Diterpene Lactone Compound from *Andrographis paniculata* and Its Interaction with Curcumin and Artesunate. Journal of Tropical Medicine, Article ID 579518.

[14] Mahdiyanti S.M. 2004. In vivo antimalarial of *Andrographis paniculata* Nees on Plasmodium berghei infected mice. Skripsi. Fakultas Farmasi Universitas Airlangga. Surabaya. (Article in Indonesian).

[15] Achmad Fuad Hafid A.F., Rifai B, Widiastuti E, Dachliyati L, Primaharinastiti R, Widyawaruyanti A. 2015. Andrographolide determination of Andrographis paniculata extracts, ethyl acetate fractions and tablets by thin layer chromatography. Journal of chemical and pharmaceutical; 7 (12), 557-561.

[16] Peter W., Portus H, and Robinson L. 1995. The four-day suppressive in vivo antimalarial test. Ann Trop Med Parasitol, 69:155–171.

[17] Widyawaruyanti A. 1999. Imunomodulator activity of diterpenoid compound of *Andrographis paniculata* Nees on the function of T-lymphocyte cytotoxicity (CD +8) mice. Thesis. Universitas Airlangga. (Article in Indonesian).

[18] Widowati R, Santa IGP, Rahman A, Indah T, Widyawaruyanti A. 2003. In vitro Antimalarial of *Andrographis paniculata* isolate againt *Plasmodium falciparum* on gametocyte stage. Majalah farmasi airlangga, vol:3, no.99-102. (Article in Indonesian).

[19] Munoz V, Sauvain M, Bourdy G, Callapa J, Bergeron S, Rojas I, *et al.* A search for natural bioactive compounds in Bolivia through a multidisciplinary approach: Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. J Ethnopharmacol 2000;69:127-37.

[20] Luo W, Liu Y, Zhang J, Luo X, Lin C, Guo J. 2013. Andrographolide inhibits the activation of NF-kappaB and MMP-9 activity in H3255 lung cancer cells. Exp Ther Med. 6(3): 743-6.

[21] Tripathi A.K., Sha W, Shulaev V, Stins M.F, Sullivan D.J. 2009. *Plasmodium falciparum*-infected erythrocytes induce NF-kappaB regulated inflammatory pathways in human cerebral endothelium. Blood, 114(19): 4243-52.

[22] Panossian A, Hovhannisyan A, Mamikonyan G, Abrahamian H, Hambardzumyan E, Gabrielian E, Goukasova G, Wikman G, Wagner H. 2000. *Pharmacokinetic and Oral Bioavailability of Andrographolide from Andrographis paniculata fixed combination Kan Jang in Rats and Human*. Phytomedicine Vol 75.

[23] Peter I.T and Anatoli V.K. 1998. The Current Global Malaria Situation, Malaria parasite Biology, Pathogenesis and Protection, ASM Press, Washington, USA.

[24] Chao W.W, Kuo Y.H, Hsieh S.L, Lin B.F. 2011. Inhibitory effects of ethyl acetate extract of Andrographis paniculata on NF-kB trans-activation activity and LPS-induced acute inflammation in mice. Evidence-based complementary and alternative medicine, pp: -9.