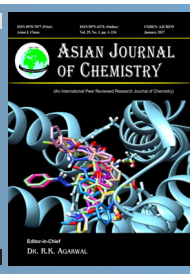
Asian Journal of Chemistry; Vol. 29, No. 3 (2017), 592-594

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2017.20268



Analysis of Some Plants Extracts Used as Antimalaria in Sei Kepayang, North Sumatera, Indonesia

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Received: 25 August 2016; *Accepted*: 18 November 2016; *Published online*: 30 December 2016; AJC-18207

In recent times, the trend in malaria research is shifting towards identifying new medicines from natural resources for management of malaria. Some Indonesian herbs have been traditionally used as antimalaria by the society in different areas of Indonesia. They include papaya leaves (*Carica papaya* L), meniran (*Phyllantus ninuri* L), pare (*Momordica charantia* L), *Smallanthus sonchifolia* and *Cassia siamea* L leaves. The antiplasmodium activities of hydro-alcoholic extracts of some standardized plant materials were screened against of *P. falciparum* strain 3D7. These results indicate the potential of antimalarial herbs.

Keywords: Medicinal plants, Standardization, Antimalaria activity.

INTRODUCTION

Malaria remains one of the world's most important health problems in both developed and developing nations. According to the World Health Organization (W.H.O.), 219 Millions of malaria cases were recorded in 2010; 660.000 of them ended up with mortality. About 90 % of the death cases occurred in Africa alone. In Asia, India accounted for 24 million of malaria-related deaths, followed by Indonesia and Myanmar [1]. Indonesia is one of the countries with high-risk of malaria incidence. The Indonesian Health Ministry reported that about 80 % of regencies/municipalities in Indonesia are malaria endemic. The health ministry also reported that malaria attacks populations, particularly in the isolated areas with poor environmental conditions, difficult transportation and communication and limited access to health services [2].

In 2009, 1.143.024 clinically cases of malaria were reported and 199.577 of them were reported to be positive according to laboratory examination. The number might be smaller than the facts since not all of the cases had been reported [2,3]. The main factor for inability to eradicate malaria is vector, namely, anopheles mosquito, which is resistant to insecticide and plasmodium, which is also resistant to the available antimalarial [3]. The research aims at making the inventory of herbs, extractions and antiplasmodial assay for 5 plants, which have been used as antimalaria in Sei Kepayang, North Sumatra. In addition, *in vitro* antimalarial assay for *P. falciparum* strain 3D7 showed that there are 3 potential plant extracts to be developed as antimalaria, including papaya (*C. papaya*) and meniran (*P. niruri* L) leaf extracts.

EXPERIMENTAL

Drug procurement and authentication: Collection of plant samples were conducted in Sungai Kepayang, Asahan Regency and terminated in the Dendrology Laboratory, Forestry Study, Faculty of Agriculture, North Sumatera University. The parts of plants to be used were washed thoroughly, chopped and desiccated to obtain simplicia.

Preparation of hydro-alcoholic extract and standardization: The extracts of simplicia were macerated using 70 % ethanol for 24 h, repeated three times, filtered and the filtrates were collected and evaporated using vacuum rotavapor to obtain thick extract. The process was followed by water bathing to obtain fixed weights. The extracts were analyzed and standardized as per API/WHO guidelines [4].

Culture of *P. falciparum*: Three solutions were required to grow *P. falciparum* that had been stored in liquid N_2 tank (Bernstead). They were solution A that consisted of 12 % NaCl, solution B that consisted of 1.60 % NaCl and solution C that consisted of 0.20 % dextrose in 0.90 % NaCl. Propagation began with dilution of frozen *P. falciparum* taken from the liquid N_2 tank and then moved into a 14 mL centrifuge flask (Nunc). Then, 200 µL of solution A was added into the flask and incubated for 2 min under room temperature, followed by addition of 100 mL of solution B and centrifugation at 3000 rpm for 5 min. The supernatants were removed and added

TABLE-1
STANDARDIZATION OF HYDROALCOHOLIC EXTRACT OF SAMPLES

Botanical name	*C. papaya*	*P. niruri*	*M. charantia*	*S. sonchifolia*	*C. siamea*
Part of the plant used	Leave	Herbs	Fruit	Leave	Leave
Loss on drying (%)	4.34	5.34	6.45	3.24	3.57
Water soluble extractive (%)	69.14	74.12	77.66	53.39	65.27
Alcohol soluble extractive (%)	75.75	46.36	69.63	69.97	72.60
Total ash (%)	9.22	2.82	8.92	5.65	0.48
Acid insoluble ash (%)	0.13	0.98	0.43	1.63	0.05
Bulk densitiy (g/mL)	0.57	0.67	0.65	0.75	0.77
Tapped density (g/mL)	1.03	1.05	1.08	0.85	0.75
Heavy metals (ppm)	< 5	< 5	< 5	< 5	< 5
Lead (ppm)	< 1	< 1	< 1	< 1	< 1
Cadmium (ppm)	< 2	< 2	< 2	< 2	< 2
Solvent residues (ethanol) (ppm)	< 500	< 500	< 500	< 500	< 500
Total flavonoid (%)	2.58	5.44	3.45	2.56	8.44
Microbiological limits (cfu/g)	< 100	< 100	< 100	< 100	< 100
Total plate count (cfu/g)	< 10	< 10	< 10	< 10	< 10
Yeast/Molds	Absent	Absent	Absent	Absent	Absent

with 100 mL of solution C, in which the deposition had been removed. *P. falciparum* infected erythrocyte deposition was cultured in a flask (Nunc) that had been filled with 8 mL of media RPMI 1640 (Sigma), 1.20 mL of Type-O human blood serum of and 50 µL of Type-O erythrocyte. The culture was grown in the flask, which was put into a candle jar. The candle was turn on, then the candle jar cap was put on in such a way that the candle gradually dimmed and eventually died out, thus producing oxygen (± 3 % O_2), carbon dioxide (± 4 % CO_2) and nitrogen (± 93 % N_2) concentrations that were suitable for plasmodium culture. When *P. falciparum* had been cultured and some would be re-stored into the liquid N_2 tank, then a freezing medium that consisted of 28 % glycerol, 3 % sorbitol and 0.65 % NaCl was necessary. The freezing solution was prepared by mixing 28 mL of glycerol and 72 mL of 4.20 % sorbitol (Sigma) in 0.90 % NaCl [5].

Material preparation: Weigh the materials to a certain level and dilute in 200 µL of DMSO and 800 µL of RPMI 1640 (Sigma) as the main solution. The solution was then diluted using RPMI and the series were prepared according to the specified concentration.

Synchronization: Synchronization was conducted to obtain the desired parasites using 5 % sorbitol solution. Then, the parasite was centrifuged for 10 min at 1000 rpm. The supernatants were eliminated; parasite deposition was immersed in the sterile sorbitol solution in 3 times of the erythrocyte volume and then kept under room temperature for 20 min. The parasite was cleansed using complete medium to obtain parasite that consisted only ring stadium [6].

Preparation of microculture: The plasmodium was cultured in 96-well microculture plate with the volume of 100 µL, 1.5 % hematocrit and 1 % parasitemia. Furthermore, 100 µL of each concentrated extracts were put into the wells using a micropipette, starting from the lowest to the highest concentrations. The microculture was grown in a candle jar and incubated in a CO_2 incubator under a temperature of 37 °C for 24 and 72 h. At the end of incubation time, thin blood smear was prepared from each well and stained using 5 % Giamsa. Parasitemia rate was determined microscopically using a 100x objective lenses and immersion oil; then, parasite count per 200 erythrocytes was determined microscopically.

***in vitro* Antiplasmodium activity:** *in vitro* Antiplasmodial assay against *P. falciparum* culture was conducted for *n*-hexane, ethyl acetate and *n*-butanol extracts with the aim of finding out antiplasmodial activity of each extract obtained and assayed. *in vitro* Assay was conducted for *P. falciparum* strain 3D7 was expected to reveal activity of the extracts against plasmodium strain. Strain 3D7 is sensitive to chloroquin.

RESULTS AND DISCUSSION

The physico-chemical analysis along with heavy metals and microbial load of dilute alcoholic extracts of natural product has been carried out (Table-1) and standardized according to standard API/WHO guidelines.

Table-2 shows that *Smallanthus sonchifolia* extract has the most potent antiplasmodial activity, compared to the other extracts (0.0178 µg/mL). Of five extracts tested, four extracts were found to have antiplasmodial activity of < 10 µg/mL. Several criteria have been proposed for considering a compound as active. Generally, a compound is considered to be inactive when it shows an IC_{50} > 200 µM, whereas those with an IC_{50} of 100-200 µM have low activity; IC_{50} of 20-100 µM, moderate activity; IC_{50} of 1-20 µM good activity; and IC_{50} < 1 µM excellent/potent antiplasmodial activity [7].

TABLE-2
PLANT PARTS COLLECTED BASED ON ETHNOPHARMACOLOGICAL DATA, PERCENTAGE YIELD DRY ETHANOLIC EXTRACT PER 100 g OF AIR-DRIED AND ANTIMALARIAL ACTIVITY ETHANOLIC EXTRACT PLANT MATERIAL USED

Botanical name (Extract)	Yield plant (%)	IC_{50} (µg/mL)
Momordica charantia (Momordicacea)	19.61	0.0178 ± 0.01
Phyllanthus niruri L (Euphorbiacea)	28.68	7.56 ± 0.54
Carica papaya L (Caricaceae)	27.41	0.177 ± 0.15
Smallanthus sonchifolia (Asteraceae)	19.75	27.45 ± 1.21
Cassia siamea (Fabaceae)	8.68	4.75 ± 0.12

Conclusion

Four of five plant extracts tested showed potent antimalarial activity, with IC_{50} value of < 10 µg/mL. Further, all these plants extract need to be screened against *in vivo* antimalaria activity to confirm the activity.

ACKNOWLEDGEMENTS

One of the authors (Syamsudin) is grateful to the Directorate General of High Education, Ministry of Education & Culture, Republic of Indonesia particularly SIMLITABMAS, for financial support received from Grand Competititon (No 0263/E5/2014).

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