IN VITRO ANTIMALARIAL ACTIVITY OF THAI *PICRASMA JAVANICA* Bl STEM EXTRACT

Chalerm Saiin[1*], Busaban Sirithunyalug[2], Roonglawan Rattanajak[3], Sumalee Kamchonwongpaisan[4], Apiwat Baramee[5], Kom Sukontason[6], Kornkanok Ingkaninan[7]

[1, 7]Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok, [2] Department of Pharmaceutical Science, Faculty of Pharmacy, Chiang Mai University, Chiang Mai, [3, 4]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Pathumthani, [5]Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, [6]Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai, THAILAND.

[1] Chalerms@nu.ac.th

ABSTRACT

The *in vitro* antimalarial activity against *Plasmodium falciparum* K1 of the four extracts from the stem of *Picrasma javanica* Bl, that is, water, methanol, chloroform and hexane were studied using a modification of the [^{3}H]hypoxanthine incorporation method. It was found the chloroform and hexane extracts showed *in vitro* antimalarial activity with IC_{50} of 21.6 μg/ml and 33.2 21.6 μg/ml, respectively. While, the methanol and water extracts were inactive against parasite. In addition, the methanol extract of leave showed the activity against *P. falciparum* K1 with IC_{50} of 21.7 μg/mL. Chemical constituent investigations of stem and leave of this medicinal plant may bring to the new lead structures for developing new antimalarial drugs.

Keywords: Antimalarial activity, *Picrasma javanica* Bl

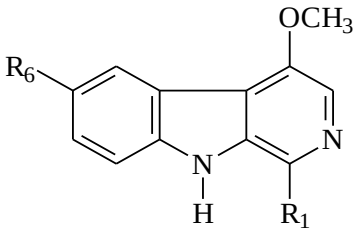
INTRODUCTION

Antimalarial drug resistance has emerged as one of the greatest challenges facing malaria control today. Resistance to antimalarial drugs has been described for two of the four species of malaria parasite that naturally infect humans, *Plasmodium falciparum* and *Plasmodium vivax*. *P falciparum* has developed resistance to nearly all antimalarials in current use, although the geographical distribution of resistance to any single antimalarial drugs varies greatly. *P. vivax* infection acquired in some areas has been shown to be resistant to chloroquine and/or primaquine (WHO, 2001). Chinese scientists have been succeeding to develop the new class antimalarial drugs, artemisinin and its derivatives, from the herb "Qinghao". This herb have been used in traditional Chinese medicine a long time ago (Tu, 2016). However, Resistance to artemisinin derivatives (ARTs) in malaria disease is currently defined as a delayed parasite clearance following artemisinin combined therapy (ACT). Although ACT is still widely effective, the first evidence of artemisinin resistance was described in 2009 in Southeast Asia (Dondorp et al., 2009). Since then, resistance to ARTs / ACT has been monitored showing an increasing trend. The demonstrated resistance to all drugs that are currently associated to ART, the ambiguous finding that ART resistance is observed only in presence of resistance to the partner drug, the lack of a mechanistic rationale to choose the partner drugs and the lack of markers with known specificity and sensitivity to monitor ART resistance, represent the most worrisome issues (Pantaleo et al., 2015). These are indicating that the new candidate antimalarial drugs shall be developed immediately.

The bark of medicinal plant *Picrasma javanica* Bl. was reputedly used for the treatment of malaria in the traditional medicine in Myanmar, Indonesia and Thailand (Old Style Doctor

Association, 1962). In 1942, during the II World War, 36 recipes of Thai Folk Medicine were used for treatment of either faciparum- or vivax infected soldiers by Dr. Ketsusinh (1948). Pavanand et al. (1988) demonstrated that the chloroform extract of the bark possessed the high level of *in vitro* antimalarial activity against *P. falciparum* asexual stage. Further isolation and purification of the chloroform extract resulted in the identification of two pure alkaloids in the class of 1-substituted-4-oxygenated-β-carbolines, 4-methoxy-1-vinyl-β-carboline and 6-hydroxy-4-methoxy-1-vinyl-β-carboline. The first compound was effective against *P. falciparum* isolates with mean IC_{50} of 2.4 μg/ml, while the second one showed mean IC_{50} of 3.2 μg/ml.

Saiin et al. (2003) reported that the *in vitro* antimalarial activities against *P. falciparum* K1 of four extracts from the stembark of *P. javanica*; ie water, methanol, chloroform and hexane extracts were studied using a modification of the [^{3}H]hypoxanthine incorporation method. It was found that the hexane extract showed in vitro antimalarial activity with IC_{50} of 3.3 microg/ml. The extract was further fractionated using quick column chromatography, resulting in ten fractions. Fraction V was the most effective against *P. falciparum* K1 with IC_{50} of 4.4 microg/ml. Further isolation of fraction V using a column chromatographic technique provided six fractions. According to ^{1}H- and ^{13}C-NMR spectra, it could be concluded that the major compound in fraction V-3 was β-sitosterol. Unfortunately, the antimalarial activity of β-sitosterol could not be determined because of its low solubility in dimethyl sulfoxide. In addition, Saiin et al. (2016) reported the results of isolation and *in vitro* antimalarial activity against *P. falciparum* K1 of chloroform extract from Thai *P. javanica* stem bark. It was found that 4-methoxy-1-vinyl-β-carboline and its transformed product 1-ethyl-4-methoxy-β-carboline play a role for antimalarial activity of *P. javanica*.



4-methoxy-1-vinyl-β-carboline: $R_1 = CH=CH_2$, $R_6 = H$

6-hydroxy-4-methoxy-1-vinyl-β-carboline: $R_1 = CH=CH_2$, $R_6 = OH$

1-ethyl-4-methoxy-β-carboline: $R_1 = CH_2CH_3$, $R_6 = H$

Figure 1 Chemical structure of 1-substituted-4-oxygenated-β-carbolines

This is the first time to report the *in vitro* antimalarial activity of *P. javanica* stem extract.

MATERIALS AND METHODS

Plant Material

Stem of *P. javanica* was collected from Queen Sirikit Botanical Garden, Chiang Mai, Thailand in July 2000, and was identified by comparing with the references deposited there, and at Faculty of Pharmacy, Chiang Mai University, Thailand.

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

Preparation of crude extracts from *P. javanica* stembark

About 100 g of dried ground *P. javanica* stem were separately macerated in 600 ml methanol, chloroform and hexane for three days or boiled with 1.5 L of water for 10 hours. Then, they were filtered and evaporated to dryness under reduced pressure. The residue plant materials were extracted again using the same process. The second extracts were pooled together with the first corresponding extractes.

In vitro antimalarial activity test

The antimalarial activity of extracts against *P. falciparum* K1 infected red cell was measured by using the [^{3}H]hypoxanthine incorporation method reported by Desjardins et al. (1979) and modified by Kamchonwongpaisan et al. (1995). Briefly, extract was dissolved in dimethyl sulfoxide and diluted with the culture medium to the required concentration. A mixture of 25 µL of the medium containing a sample and 200 µL of 1.5 % cell suspension with 1-2 % parasitemia at ring stage was cultured for 24 h, after which 25 µL of 0.25 µCi [^{3}H]hypoxanthine was added. After addition at 18 h in culture, the cells were harvested onto glass-fiber filters (Unifilter®, Packard, USA). The filters were air-dried and 20 µL liquid scintillation fluid (Microscint, Packard) was added. The radioactivity on the filters was then measured using a microplate scintillation counter (Topcount, Packard, USA). The IC_{50}s, the concentrations required for 50 % reduction of the radioactivity as compared to control without the sample, of the sample against these infected cells were obtained from dose-response curves.

RESULTS AND DISCUSSIONS

In vitro antimalarial activity of *P. javanica* stem extracts

The four crude extracts of *P. javanica* stem were tested for *in vitro* antimalarial activities against *P. falciparum* K1 by using the [3H]hypoxanthine incorporation method. The chloroform extract of stem showed the activity against *P. falciparum* K1 with IC_{50} of 21.6 μg/mL (Table 1), which was comparable to that of the chloroform extract of stembark (IC_{50} of 20.0 μg/mL (Saiin et al., 2003). Interestingly, the methanol extract of stem was inactive against *P. falciparum* K1, while the methanol extract of stembark and leave showed the activity against *P. falciparum* K1 with IC_{50} of 22.1 μg/mL and 21.7 μg/mL, repectively (Table 2).

Table 1. Crude extracts obtained from *P. javanica* stem and their antimalarial activities against *P. falciparum* K1.

Crude extracts	*Weight (g)*	*% yield*	*IC_{50} against P. falciparum K1 (μg/ml)*
Methanol crude extract	1.634	1.35	Inactive
Chloroform crude extract	0.254	0.24	21.6
n-Hexane crude extract	0.157	0.15	33.2
Water crude extract	3.819	3.42	Inactive

Table 2. Comparision the antimalarial activities against *P. falciparum* K1 of the methanol extract of *P. javanica* stem, stembark, and leave

Part used	*Weight of crude extract (g)*	*% yield*	*IC_{50} against P. falciparum K1 (μg/ml)*
Stem	1.634	1.35	Inactive
Stembark*	3.674	2.84	22.1
Leave**	no record	no record	21.7

* Saiin et al. (2003)

**preparation of crude extract and antimalarial activity test performed in the same method with stem

Although, sixteen indo-type-alkaloids were reported for *P. javanica*, only Javacarboline was purified from the stem of this plant (Johns et al., 1970, Arbain and Sargent, 1987, Ohmoto et al., 1987, Pavanand et al., 1988, Yoshikawa et al., 1993, Koike et al., 1994, Saiin et al., 2016). Koike et al. (1994) studied the chemical constituent of stem of *P. javanica* and reported the new compound, Javacarboline. The indo-type-alkaloid, Javacarboline that contain the β-carboline moiety may respond for the antimalarial activity of *P. javanica* stem extract in the same manner with other β-carbolines as reported by Takasu et al. (2004 and 2005). The purposed mechamism of action of β-carbolines as antimalarial agents based on π-Delocalized Lipophilic Cations (Takasu, 2016). In another hand, there may be other indo-type-alkaloids played the role for antimalarial activity of the stem of *P javanica*.

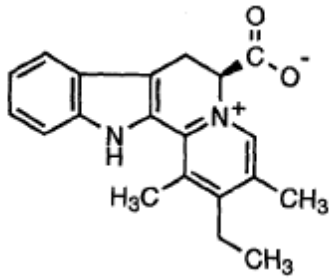


Figure 2 Chemical structure of Javacarboline

In vitro antimalarial activity of *P. javanica* leave extract

In addition, the antimalarial activity of the methanol extract of the leave corresponded with the result of Indonesia *P javanica*. In that study, *P. javanica* extracts was tested for antimalarial against rodent plasmodium, *Plasmodium berghei* in single dose (20 mg/kg BW). The results showed that stembark and leave extracts decrease parasitemia 73.89% and 71.4%, respectively, and chloroquine (32.35%) as positive control (Praptiwi et al., 2007).

CONCLUSION

It could be concluded that the stem and leave of *P. javanica* compose of the antimalarial compounds. Chemical constituent investigations of stem and leave of this medicinal plant may bring to the new lead structures for developing new antimalarial drugs.

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp

REFERENCES

[1] Arbain, D., & Sargent, M.V. (1987). The alkaloids of picrasma javanica. *Aust. J. chem., 9*, 1527-1536.

[2] Desjardins, R. E., Canfield, C. J., Haynes, D. E., & Chulay, J.D. (1979). Quantitative assessment of antimalarial activity *in vitro* by a semiautomated microdilution technique. *Anti-microb Agents Chemotherapy, 16*, 710-718.

[3] Dondorp, A. M., Nosten, F., Yi, P., Das, D., Phyo, A.P., Tarning, J., Lwin, K.M., Ariey, F., Hanpithakpong, W., Lee, S.J., Ringwald, P., Silamut, K., Imwong, M., Chotivanich, K., Lim, P., Herdman, T., An, S.S., Yeung, S., Singhasivanon, P., Day, N.P., Lindegardh, N., Socheat, D., & White, N.J. (2009). Artemisinin resistance in plasmodium falciparum malaria. *N Engl J Med., 361*(5), 455-67.

[4] Johns, S. R., Lamberton, J. A., & Sioumis, A. A. (1970). 4-Methoxy-1-vinyl-β-carboline, a new alkaloid from picrasma javanica (Simaroubaceae). *Aust. J. Chem., 23*, 629-630.

[5] Kamchonwongpaisan, S., Paitayatat, S., Thebtaranonth, Y., Wilairat, P. & Yuthavong, Y. (1995). Mechanism-based development of new anti-malarials: Synthesis of derivatives of artemisinin attached to iron chelators. *J. Med. Chem., 38*, 2311-2316.

[6] Ketusinh, O. (1948). *Report on clinical antimalarial therapy of Thai medicinal plants*. USA: Proceeding of the Siriraj 60th Aniversary Meeting.

[7] Koike, K., Ohmoto, T., Uchida, A., & Onishi, I. (1994). Javacarboline, A new β-carboline alkaloid from the stem of picrasma javanica in Java. *Heterocycles, 38*(6), 1413-1420.

[8] Ohmoto, T., Koike, K., & Mitssunaga, K. (1987). Alkaloids from picrasma javanica growing in Indonesia. *Shoyakugaku Zasshi*, *41*(4), 338-340.

[9] Old-style Doctor Association. (1962). *Pra-muan-sappa-kun-ya-thai* (Part I). Bangkok: Amphonpittaya.

[10] Pantaleo, A., Pau, M.C., Chien, H.D., & Turrini, F. (2015). Artemisinin resistance, some facts and opinions. *J Infect Dev Ctries., 9*(6), 597-9.

[11] Pavanand, K., Yongvanitchit, K., Webster, H. K., Dechatiwongse, T., Nutakul, W., Jewvachdamrongkul, Y., & Bansiddhi, J. (1988). Antimalarial activity of a Thai medicinal plant picrasma javanica. *Bl. Phytother. Res., 2*, 33-36.

[12] Praptiwi, M., & Chairul, H. (2007). Anti-malaria in-vivo activity test of ki pahit extract (picrasma javanica) to mice infected with plasmodium berghei. *Biodiversitas, 8*(2)111-113.

[13] Saiin, C., Rattanajak, R., Kamchonwongpaisan, S., Ingkaninan, K., Sukontason, K., Baramee, A., & Sirithunyalug, B. (2003). Isolation and in vitro antimalarial activity of hexane extract from Thai picrasma javanica Bl stembark. *Southeast Asian J Trop Med Public Health, 34* (2), 51-55.

[14] Saiin, C., Sirithunyalug, B., Rattanajak, R., Kamchonwongpaisan, S., Ingkaninan, K., Baramee, A., & Sukontason, K. (2016). Isolation and in vitro antimalarial activity of chloroform extract from Thai picrasma javanica Bl stembark. *Adv med plant Res, 4*(3), 94-98.

[15] Takasu, K., Shimogama, T., Saiin, C., Kim, H-S., Wataya, Y., & Ihara, M. (2004). π–delocalized β–carbolinium cations as potential antimalarials. *Bioorg. Med. Chem. Lett., 14*,1689-1692.

[16] Takasu, K., Shimogama, T., Saiin, C., Kim, H.S., Wataya, Y., Brun, R., & Ihara, M. (2005). Synthesis and evaluation of beta-carbolinium cations as new antimalarial agents based on pi-delocalized lipophilic cation (DLC) hypothesis. *Chem Pharm Bull, 53* (6), 653-61.

[17] Takasu, K. (2016). π-delocalized lipophilic cations as new candidates for antimalarial, antitrypanosomal and antileishmanial agents: Synthesis, evaluation of antiprotozoal potency, and insight into their action mechanisms. *Chem Pharm Bull, 64*(7), 656-67.

[18] Tu, Y. (2016). Artemisinin-A gift from traditional Chinese medicine to the world (Nobel lecture). *Angew Chem Int Ed Engl., 55*(35), 10210-26.

[19] World Health Organization (WHO). (2001). *Drug resistance in malarial*. Retrieved from http://www.who.int/crs/resources/publications/drugresist/malaria.pdf.

[20] Yoshikawa, M., Harada, E., Aoki, S., Yamahara, J., Murakami, N., Shibuya, H., & Kitagawa, I. (1993). Indonesia medicinal plants. VI. on the chemical constituents of the bark of picrasma javanica Bl (Simaroubaceae) from Flores island: Absolute stereo structures of picra javanins A and B. *Chem Pharm Bull, 41*(12), 2101-2105.

Leena and Luna International, Chikusei, Japan.
(株) リナアンドルナインターナショナル, 筑西市,日本
ISSN: 2186-8476, ISSN: 2186-8468 Print
www.ajsc. leena-luna.co.jp