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Isolation and *in vitro* antimalarial activity of chloroform extract from Thai *Picrasma javanica* Bl stembark

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ABSTRACT

The *in vitro* antimalarial activity against *Plasmodium falciparum* K1 of the four extracts from the stembark 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 extract showed *in vitro* antimalarial activity with IC_{50} of 20.0 µg/ml. The extract was further isolated using acid-base solvent extraction method, yielding alkaloidal and non-alkaloidal portion. The alkaloidal portion was effective against *P. falciparum* with *in vitro* IC_{50} of 15.0 µg/ml. The component of alkaloidal portion was determined using GC-MS technique. Mass spectrum of the major compound in alkaloidal portion corresponds to that of known antimalarial 4-methoxy-1-vinyl-β-carboline. Further purification using preparative TLC, the structure of this alkaloid was transformed to potential antimalarial Crenatine (1-ethyl-4-methoxy-β-carboline). According to the literature review data, further study should be focus on the antimalarial activity of sixteen indoalkaloids reported for this medicinal plant.

Keywords: β-carboline, antimalarial activity, *Picrasma javanica* Bl, 4-methoxy-1-vinyl-β-carboline, 1-ethyl-4-methoxy-β-carboline.

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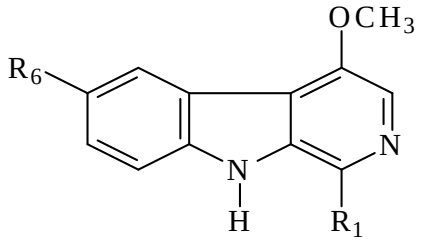
INTRODUCTION

According to the latest estimates from WHO, there were 214 million new cases of malaria worldwide in 2015 (range 149 to 303 million). The African Region accounted for most global cases of malaria (88%), followed by the South-East Asia Region (10%) and the Eastern Mediterranean Region (2%). In 2015, there were an estimated 438,000 malaria deaths (range 236,000 to 635,000) worldwide. Most of these deaths occurred in the African Region (90%), followed by the South-East Asia Region (7%) and the Eastern Mediterranean Region (2%) (WHO, 2015). Human malaria is caused by four species of parasitic protozoa (i) *Plasmodium falciparum* We., (ii) *P. vivax* Gr. and Fe., (iii) *P. ovale* St. and (iv) *P. malariae* Gr. and Fe. Of the four malaria species mentioned, *P. falciparum* is responsible for the most severe and deadly form of malaria. However, there are only a few antimalarial drugs available for the treatment of *P. falciparum* infection. In addition, the resistance of *P. falciparum* to the classical antimalarial drugs such as quinine, chloroquine and mefloquine, is rapidly increased (Willems, 1991; Bray and Ward, 1993; Nosten and Price, 1995; Olliaro et al., 1996). These have triggered off a massive screening of new compounds from either synthesis or natural products for potential antimalarials.

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. Ketusinh (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 (Figure 1). 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*; that is, 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 IC50 of 3.3 μg/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 IC50 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.

Herein, the results of isolation and *in vitro* antimalarial activity of the chloroform extract from Thai *P. javanica* stembark are reported. Also, the perspective for the further study of this medicinal plant is discussed.



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.

MATERIALS AND METHODS

Plant material

Stembark 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.

Preparation of crude extracts from *P. javanica* stembark

About 100 g of dried ground *P. javanica* stembark were separately macerated in 600 ml methanol, chloroform and hexane for three days or boiled with 1.5 L of water for 10 h. 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 extracts (Saiin et al., 2003).

Preparation of chloroform crude extract

About 1628.33 g of dried ground *P. javanica* stembark was macerated in 4 L of chloroform for seven days. Then, it was filtered and evaporated to dryness under reduced pressure. The residue plant material was extracted again using the same process. The second extract was pooled together with the first extract.

Isolation of chloroform crude extract

21.901 g of the chloroform crude extract was dissolved in chloroform (100 ml) and extracted with 2% sulfuric acid (5 × 100 ml). The combined acid extract was made alkaline with 25% ammonia solution and repeatedly extracted with five portion of chloroform (400, 4 × 200 ml). The combined chloroform extract was washed with water (2 × 100 ml) dried with anhydrous sodium sulfate, filtered and dried under reduced pressure to provide an alkaloidal portion compose of 4-methoxy-1-vinyl-β-carboline.

Further purification using preparative TLC was performed on 5 mm coated of silica gel 60 245F, 20 × 20 glass plate using n-hexane – ethyl acetate (7:3) as mobile phase

Structure elucidation of pure compound in alkaloidal portion

The chemical structure of pure compound in alkaloidal portion was elucidated from mass spectra and also compared with the data from literature. GC chromatogram and mass spectra of extracts were carried on Varian GC Star 3400CX coupled with Varian SATRUM 2000 GC-MS system using J&W Scientific 30 m × 0.251 mm DB-5MS column with 0.25 μm film thickness and helium as carrier gas. The temperatures of the GC-MS instrument were set as 280°C at the injector, 260°C at the transfer line and GC oven was programmed as followed: the temperature was initially held at 100 °C for 1 min, increased at 16.6°C/min to 150°C and held for 1 min, increased at 22°C/min to 260°C and held for 15 min. The first run, mass analysis parameters was set for electron impact (EI) mode. The second run, the holding time at 260°C was decreased to 10 min and mass analysis parameter was set for chemical ionization (CI) mode using methane as reagent gas.

In vitro anti-malarial 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 to 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 concentration 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 DISCUSSION

Extraction, isolation and *in vitro* antimalarial activity

The extraction of dried ground stembark of Thai *P. javanica* provided about 23 g (1.41%) of chloroform crude extract (Table 1). Further isolation of 21.90 g of chloroform crude extract using acid-base extraction method provided alkaloidal portion 17.5 mg (0.08%) and non-alkaloidal portion about 19 g (86.75%). The *in vitro* antimalarial activity test of these portion showed that alkaloidal portion was effective against *P. falciparum* K1 with IC_{50} of 15.0 μg/ml, while non-alkaloidal portion showed IC_{50} of 22.0 μg/ml (Table 2).

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

Crude extracts	Weight (g)	% yield	IC_{50} against *P. falciparum* K1 (μg/ml)
Methanol crude extract	3.674	2.84	22.1
Chloroform crude extract	1.674	1.61	20.0
n-Hexane crude extract	0.453	0.38	3.3
Water crude extract	10.487	9.03	Inactive

Table 2. Isolated portions obtained from chloroform extract of *P. javanica* stembark and their antimalarial activities against *P. falciparum* K1.

Isolated portions	Weight (g)	% yield	IC_{50} against *P. falciparum* K1 (μg/ml)
Alkaloid portion	0.0175	0.08	15.0
Non-alkaloid portion	19	86.75	22.0

Structure elucidation of pure compound

The GC chromatogram of alkaloidal portion showed the major peak of pure compound at the retention time of 13.14 min. The EI mass spectrum of the major GC peak showed the important peaks at *m/z* 224 (100%), 209 (14%), 181 (39%), 154 (19%) and 126 (15%). The CI mass spectrum of the major GC peak (retention time of 13.03 min) showed the main two peaks at *m/z* 225 (100%) and 253 (29%). According to the mass spectra, the pure compound of alkaloidal portion could be assigned as a known antimalarial 4-methoxy-1-vinyl-β-carboline (M). The EI mass spectrum showed the molecular ion peak ($M^{\bullet+}$) at *m/z* 224. The peaks at *m/z* 209, 181 and 154 represented for fragment ions $M^{\bullet+}$- $CH_3^{\bullet}$, $M^{\bullet+}$- $CH_3^{\bullet}$- C_2H_4 and $M^{\bullet+}$- $CH_3^{\bullet}$- C_2H_4 – CHN, respectively. The CI mass spectrum showed the M + H^+ ion peak at *m/z* 225. The peak at *m/z* 253 represented M + $C_2H_5^+$ ion. In addition, the EI mass spectrum was in accordance with published spectrum of 4-methoxy-1-vinyl

Table 3. Indoalkaloids reported for *P. javanica* Bl.

Compounds	Parts	Sources	References
Canthin-6-one	Bark	Indonesia	Ohmoto et al. (1987)
1-Acetyl-4-methoxy-β-carboline	Bark	Indonesia	Yoshikawa et al. (1993)
1-Ethyl-4-methoxy-β-carboline	Bark	Indonesia	Yoshikawa et al. (1993)
1-Ethyl-β-carboline	Bark	Indonesia	Ohmoto et al. (1987)
4-Methoxy-1-vinyl-β-carboline	Bark	Australia	Johns et al. (1970)
	Bark	Indonesia	Yoshikawa et al. (1993)
	Stembark	Thailand	Pavanand et al. (1988)
6-Hydroxy-4-methoxy-1-vinyly-β-carboline	Stembark	Thailand	Pavanand et al. (1988)
Crenatidine	Bark	Indonesia	Ohmoto et al. (1987)
Crenatine	Bark	Indonesia	Arbain and Sargent (1987)
5-Hydroxycrenatine	Bark	Indonesia	Arbain and Sargent (1987)
Dehydrocrenatine	Bark	Indonesia	Arbain and Sargent (1987)
5-Hydroxy dehydrocrenatine	Bark	Indonesia	Arbain and Sargent (1987)
Javacarboline	Stem	Indonesia	Koike et al. (1994)
Picascidine J	Bark	Indonesia	Ohmoto et al. (1987)
Picascidine G	Bark	Indonesia	Yoshikawa et al. (1993)
Picascidine I	Bark	Indonesia	Ohmoto et al. (1987)
Picascidine T	Bark	Indonesia	Ohmoto et al. (1987)

-β-carboline (Pavanand et al., 1988). Further purification of alkaloidal portion using preparative TLC was performed on 5 mm coated of silica gel 60 254F, 20 × 20 glass plate using *n*-hexane-ethyl acetate (7-3) as mobile phase. The result, however, provided only small amount of transformed product; 1-ethyl-4-methoxy-β-carboline showed molecular ion peak at m/z (CI) 226.

The perspective for the antimalarial activity of *P. javanica*

It is noteworthy that, the synthesized 1-ethyl-4-methoxy-β-carboline was effective against *P. falciparum* (chloroquine sensitive strain FCR-3) with an EC_{50} of 1.6×10^{-5} M and cytotoxic to mouse mammary tumor FM3A cell with an EC_{50} of 1.8×10^{-5} M (Takasu et al., 2004). Its selective toxicity (EC_{50} value for FM3A/EC_{50} for *P. falciparum*) was only 1.1, while quinine selective toxicity was 910. Moreover, sixteen indoalkaloids were reported for *P. javanica* (Table 3). The further study should be focus on the antimalarial activity of these indoalkaloids.

CONCLUSION

In order to study structure-activity relationships of 1-substituted-4-oxygenated-β-carbolines, the antimalarial activities and chemical constituents of *P. javanica* were reinvestigated. Our result demonstrated that 4-methoxy-1-vinyl-β-carboline and 1-ethyl-4-methoxy-β-carboline play a role for antimalarial activity of *P. javanica*. The further study should be focus on the antimalarial activity of indoalkaloids of this medicinal plant.

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