Advancement in Medicinal Plant Research
Vol. 7(2), pp. 61-67, June 2019
DOI: 10.30918/AMPR.72.19.025
ISSN: 2354-2152
Full Length Research Paper

Determination of β-carbolines in Thai *Picrasma javanica* Bl.; the source of potential antimalarial agents

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Accepted 14 June, 2019

ABSTRACT

The bark of *Picrasma javanica* Bl. (Family Simaroubaceae) has been widely used in the traditional medicines for the treatment of malaria in Myanmar, Indonesia and Thailand. In previous studies, thirteen β-carbolines were isolated from *P. javanica* growing in Indonesia and New Guinea. The present study aimed to identify the chemical components of Thai *P. javanica* by using thin layer chromatography (TLC) and high resolution mass spectrometry (MS). It demonstrated that there were at least six β-carbolines in stem bark of Thai *P. javanica*; composed of 1-ethyl-β-carboline, 1-ethyl-4-methoxy-β-carboline (crenatine), 4-methoxy-1-vinyl-β-carboline (dehydrocrenatine), 5- or 6- or 8-dehydrocrenatine, 5- or 8-hydroxycrenatine, picrasidine G and picrasidine T. Three of them i.e. crenatine, dehydrocrenatine and 6-dehydrocrenatine had the antimalarial activities. Thus, Thai *P. javanica* shall be reserved as medicinal plant for malaria disease treatment.

Keywords: Thai *Picrasma javanica* Bl., Indole alkaloids, thin layer chromatography, mass spectrometry.

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INTRODUCTION

Picrasma javanica Bl. is a medium size tree in the family Simaroubaceae. It's bark has been used in traditional medicines for the treatment of malaria in Myanmar, Indonesia and Thailand (Old Style Doctor Association, 1962). In 1942, during the II World War, 36 recipes of Thai Folk Medicine included *P. javanica* were used for treatment of either *Plasmodium falciparum* or *P. vivax* infected soldiers by Ketusinh (1948). Tangjitman et al. (2013) compared traditionally medicinal knowledge in 14 Karen villages in northern Thailand and determined culturally important medicinal plant in each Karen villages. *P. javanica* showed cultural important index (CI) of 0.96. Therefore, the chemical constituents and pharmacological activities of *P. javanica* should be studied thoroughly in order to justify the efficacy and safety of *P. javanica* as antimalarial drug among the Karen in Thailand.

Saiin and Sirithunyalug (2017) reviewed the chemical structure of β-carbolines isolated from *P. javanica*. Most of fourteen indole alkaloids (Figure 1) were isolated from *P. javanica* growing in Indonesia and New Guinea. In Thailand, 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, 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 LC_{50} of 2.4 μg/ml, while the second one showed mean LC_{50} of 3.2 μg/ml.

Moreover, several β-carbolines including naturally

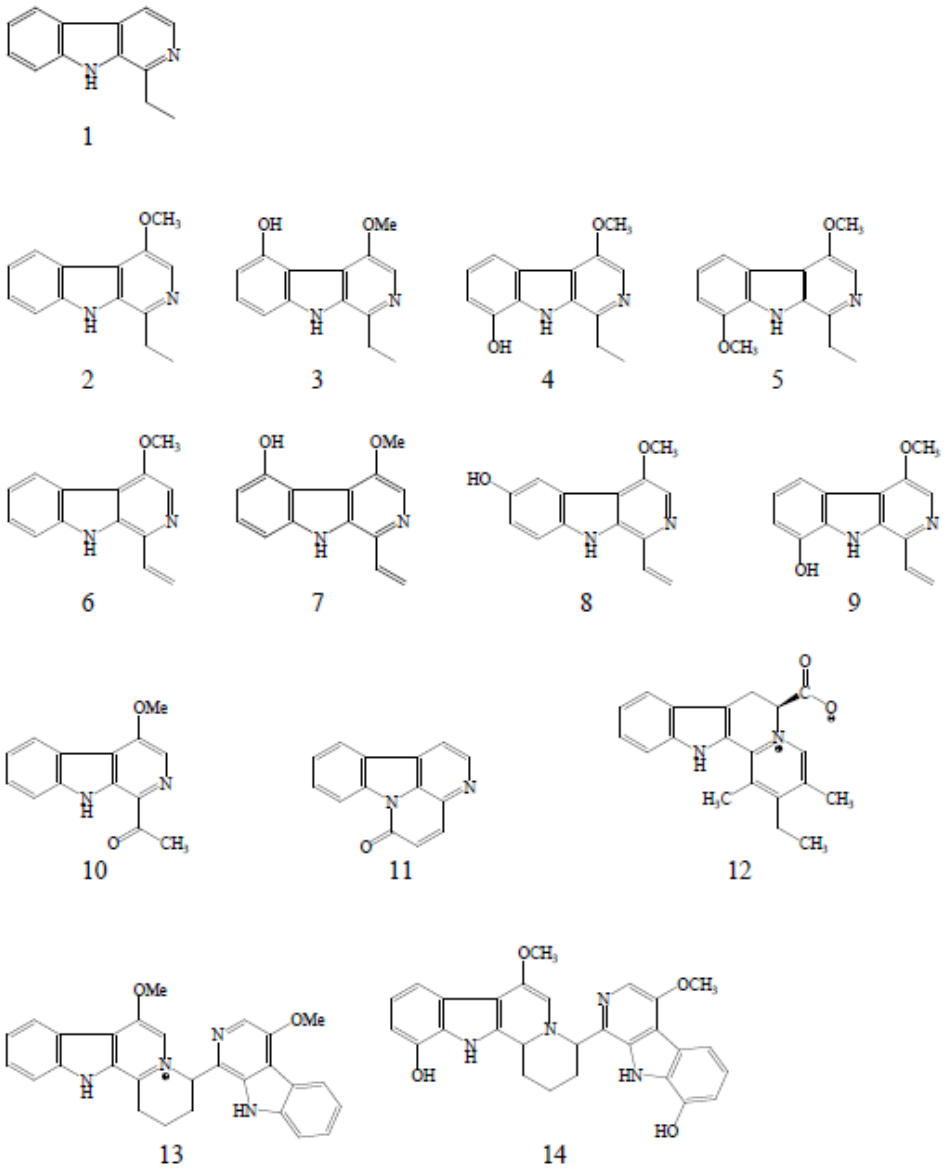

Figure 1. Chemical structures of fourteen indole alkaloids isolated from *P. javanica* reported by Johns et al. (1970), Arbain and Sargent (1987), Ohmoto et al. (1987), Pavanand et al. (1988), Yoshikawa et al. (1993), Koike et al. (1994): (1); 1-Ethyl-β-carboline, (2); Crenatine [1-Ethyl-4-methoxy-β-carboline], (3); 5-Hydroxycrenatine [5-hydroxy-4-methoxy-β-carboline], (4); Picrasidine J [8-hydroxy-4-methoxy-β-carboline], (5); Crenatidine [4, 8-dimethoxy-β-carboline], (6); Dehydrocrenatine [4-methoxy-1-vinyl-β-carboline], (7); 5-Hydroxydehydrocrenatine [5-Hydroxy-4-methoxy-1-vinyl-β-carboline], (8); 6-Hydroxy-4-methoxy-1-vinyl-β-carboline, (9); Picrasidine I [8-hydroxy-4-methoxy-1-vinyl-β-carboline], (10); 1-Acetyl-4-methoxy-β-carboline, (11); Canthin-6-one, (12); Javacarboline, (13); Picrasidine G and (14); Picrasidine T.

occurring substances and their corresponding cationic derivatives were synthesized and evaluated for antiplasmodial activity. It was found that dehydrocrenatine (4-methoxy-1-vinyl-β-carboline) was effective against *P. falciparum* chloroquine sensitive strain (FCR-3) with the EC_{50} of 5.0×10^{-6} M, while crenatine (1-ethyl-4-methoxy-β-carboline) showed as EC_{50} of 1.6×10^{-5} M (Takasu et al., 2004, 2005). Importantly, their salts; quaternary carbolinium cations not only showed much higher potencies than neutral β-carbolines, but also could increase selectivity between efficacy and toxicity.

This research aimed to determine the β-carbolines, subclass of indole alkaloids, in Thai *P. javanica* by using thin layer chromatography (TLC) and high resolution mass spectrometry (MS).

MATERIALS AND METHODS

Plant material

The stem bark of *P. javanica* collected from Queen Sirikit Botanic Garden, Chiang Mai and was identified by comparison with the references deposited there (15 years).

Preparation of crude extracts from *P. javanica* stem bark

The dried ground stem bark of *P. javanica* was macerated with hexane and dried in oven (60°C). It was packed in plastic bag and kept at room temperature. In this study, about 100 g of dried ground hexane extracted *P. javanica* stem bark were successively extracted with chloroform and methanol. The fine ground and rough ground stem bark were macerated in 500 ml chloroform and methanol for three days. 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.

TLC system

TLC aluminum sheet coated with silica gel GF 254 (Merck) was used as stationary phase. Mobile phase were chloroform-methanol-water (7:3:1), ethyl acetate (1:0), ethyl acetate-ethanol (8:2), ethyl acetate-ethanol (8:1), ethyl acetate-ethanol (8:2), ethyl acetate-ethanol (9.5:0.5), ethyl acetate-ethanol (9.9:0.1). Alkaloids were identified by UV light (254 nm) and color spot test with major alkaloidal test solutions; Wagner's reagent and Erdman's reagent (Soonthornchareonnon et al., 2008). 1-Ethyl-β-carboline (Junwised et al., 2019), 1-ethyl-1,2,3,4-tetrahydro-β-carboline (Saiin et al., 2018) and tiliacorinine (Saiin and Markmee, 2003) were used as standard alkaloids.

High resolution mass spectrometry

The high resolution mass spectrometry (MS) were taken with Agilent technologies; 6540 UHD Accurate mass QTOF LC/MS. Sample solution (10 µg/ml methanol, 1 µl) was taken directly to MS and used positive charge electro spray ionization mode (+ESI).

RESULTS AND DISCUSSION

Successively extraction of *P. javanica* stem bark

In order to wash the β-sitosterol and others lipophilic components, the stem bark was extracted with hexane. Then, the hexane extracted stem bark was extracted with chloroform. Finally, the chloroform extracted stem bark was extracted with methanol. The solution of methanol extract was taken directly to mass spectrometry.

It was found that the fine ground stem bark gave higher yield than rough ground stem bark both successively extraction with chloroform and methanol as showed in Table 1. It could be resulted from β-sitosterol; major compound in hexane extract (Saiin et al., 2003). Fine ground resulted in broken the cell and increase the area of the stem bark, thus β-sitosterol and others lipophilic components could be dissolved well.

TLC chromatogram

Various solvent systems were used as mobile phase for isolation of alkaloids in extracts. It was found that chloroform-methanol-water (7:3:1) and ethyl acetate-ethanol [(1:0), (8:2), (9:1), (9.5:0.5) and (9.9:0.1)] provided good separation. Wagner's reagent gave reddish-brown when reacted with the alkaloids in extracts and standards; 1-ethyl-β-carboline, 1-ethyl-1,2,3,4-tetrahydro-β-carboline and tiliacorinine. Erdmann's reagent gave deep reddish-brown when reacted with alkaloids in extracts, 1-ethyl-β-carboline, 1-ethyl-1,2,3,4-tetrahydro-β-carboline, but gave green color when reacted with tiliacorinine. Retardation factor (Rf) was calculated as showed in Table 2. 1-Ethyl-β-carboline was one of fourteen indole alkaloids isolated from *P. javanica*. Synthesized 1-ethyl-β-carboline and its derivative 1-ethyl-1,2,3,4-tetrahydro-β-carboline were used to estimate the Rf value of other alkaloids in the extracts. Tiliacorinine was used to confirm that the reagents were reacted with alkaloids and molecular weight and polarity of tiliacorinine should similar with picrastidine G and picrasidine T.

According to TLC chromatogram, the number of alkaloids found in the fine ground extracts were lower than the number of alkaloids found in the rough ground extracts as shown in Table 2.It might result from the interference effect of the lipophilic component in the fine ground extracts. In addition, chloroform extract gave smaller amount of alkaloids than methanol extract. These results corresponded to the report of Otmoto et al. (1987) who successively extracted the air dried bark of *P. javanica* growing in Indonesia with *n*-hexane, chloroform, and then methanol. The chloroform extract gave very low yield of crystal1-ethyl-β-carboline, crenatine, picrasidine I, crenatidine, and canthin-6-one. While the methanol extract gave higher yield of picrasidine I, picrasidine J, and crude picrasidine T.

High resolution mass spectrum

The methanol extract of rough ground was analyzed by using high resolution mass spectrometry as shown in Figure 2. The peak at *m/z*197.1074 $[M+H]^+$could be 1-ethyl-β-carboline. The peak at *m/z*225.1035 $[M+H]^+$ could

Table 1. Crude extracts obtained from *P. javanica* stem bark.

Crude extracts	Weights	% yield
Chloroform crude extract of fine ground stem bark (KC1)	4.3892	4.39
Methanol crude extract of fine ground stem bark (KM1)	3.2528	3.48
Chloroform crude extract of rough ground stem bark (KC2)	1.7563	1.76
Methanol crude extract of rough ground stem bark (KM2)	1.9099	1.99

Table 2. Rf value of alkaloids found in the extract of *P. javanica* stem bark.

Mobile phase	Detection	Rf value							Number of alkaloid
		KC1	KC2	KM1	KM2	Std1	Std2	Std3	
Chroloform : methanol : water (7:3:1)	Ultraviolet-Visible	0.27 0.91	0.27 0.91	0.29 0.91	0.29 0.81	0.89	0.27	0.27	4
	Wagner's reagent	-	-	-	-	0.89	0.27	0.27	-
Ethyl acetate (1:0)	Ultraviolet-Visible	0.03 0.60 0.76 0.92	0.03 0.60 0.76 0.92	0.03 0.60 0.76 0.92	0.03 0.60 0.76 0.92	0.5	0.03	0.86	4
	Wagner's reagent	0.03 0.92	0.03 0.92	0.03 0.92	0.03 0.92	0.5	0.03	0.86	2
	Ultraviolet-Visible	0.10 0.89 0.92	0.10 0.89 0.92	0.14 0.89 0.92	0.14 0.30 0.89 0.92	0.88	0.14	0.92	5
Ethyl acetate : Ethanol (8:2)	Wagner's reagent	0.10 0.89 0.92	0.10 0.89 0.92	0.14 0.89 0.92	0.14 0.30 0.89 0.92	0.88	0.14	0.92	5
	Erdman's reagent	0.10 0.89 0.92	0.10 0.89 0.92	0.14 0.89 0.92	0.14 0.30 0.89 0.92	0.88	0.14	0.92	5
Ethyl acetate : Ethanol (9:1)	Ultraviolet-Visible	0.03 0.06 0.82 0.91	0.03 0.06 0.82 0.91	0.03 0.82 0.91	0.03 0.06 0.23 0.82 0.91	0.76	0.11	0.93	5
	Wagner's reagent	0.03 0.06 0.82 0.91	0.03 0.06 0.82 0.91	0.03	0.03 0.06 0.82	0.76	0.11	0.93	5
	Erdman's reagent	-	-	-	0.03	-	0.11	-	-

Table 2. Continues.

Ethyl acetate : Ethanol (9.5:0.5)	Ultraviolet-Visible (conc.)	0.03 0.06 0.74 0.91	0.03 0.06 0.74 0.91	0.03 0.74 0.91	0.03 0.06 0.23 0.74 0.91	0.70	0.11	0.94	5
	Iodine vapour	0.03 0.06 0.74 0.91	0.03 0.06 0.74 0.91	0.03 0.74 0.91	0.03 0.06 0.23 0.74 0.91	0.70	0.11	0.94	5
	Wagner's reagent	0.03 0.06	0.03 0.06	0.03	0.03 0.06	0.88	0.11	-	2
	Erdman's reagent	0.03 0.91	0.03 0.91	0.03 0.91	0.03 0.91	0.70	0.11	0.94	2
Ethyl acetate : Ethanol (9.9:0.1)	Ultraviolet-Visible (conc.)	0.03 0.63 0.82 0.91	0.03 0.63 0.82 0.91	0.03 0.12 0.63 0.82 0.91	0.03 0.12 0.63 0.82 0.91	0.73	0.11	0.92	5
	Iodine vapour	0.06 0.82 0.91	0.03 0.82 0.91	0.03 0.82 0.91	0.03 0.06 0.82 0.91	0.73	0.11	-	4
	Wagner's reagent	0.03 0.82 0.91	0.03 0.82 0.91	0.03 0.82 0.91	0.03 0.06 0.82 0.91	0.73	0.11	-	4
	Erdman's reagent	0.03	0.03	0.03	0.03	0.73	0.11	-	1

KC1 = Chloroform crude extract of fine ground stem bark, KM1= Methanol crude extract of fine ground stem bark, KC2= Chloroform crude extract of rough ground stem bark, KM2= Methanol crude extract of rough ground stem bark, Std1= 1-ethyl-β-carboline, Std2= Tiliacorinine, Std3= 1-ethyl-1,2,3,4-tetrahydro-β-carboline.

be crenatine. The peak at *m/z* 227.1184 $[M+H]^+$ could be dehydrocrenatine. The peak at *m/z* 241.0981 $[M+H]^+$ could be 5- or 6- or 8- hydroxydehydrocrenatine. The peak at *m/z* 243.1134 $[M+H]^+$ could be 5- or 8-hydroxycrenatine. The peak at *m/z* 449.1986 $[M+H]^+$ could be picrasidine G. The peak at *m/z* 481.1886 $[M]^+$ could be picrasidine T. According to reviewing the structure of indole alkaloids isolated from *P. javanica* by Saiin and Sirithunyalug (2017), it was found two suspects. First, 6-hydroxydehydrocrenatine (6-hydroxy-4-methoxy-1-vinyl-β-carboline) reported by Pavanand et al. (1988) may be 5-hydroxydehydrocrenatine as reported by Arbain and Sargent (1987). Second, picrastidine T hydrochloride (m/z 448 [M-HCl]) reported by Ohmoto et al. (1987) must be picrastidine G hydrochloride. In addition, the present findings reported the peak at m/z 481.1886 that should be picrastidine T.

Comparison the β-carbolines found in Thai and Indonesian *P. javanica*

According to the TLC data, high resolution MS data and literature review data, it is shown that Thai *P. javanica*

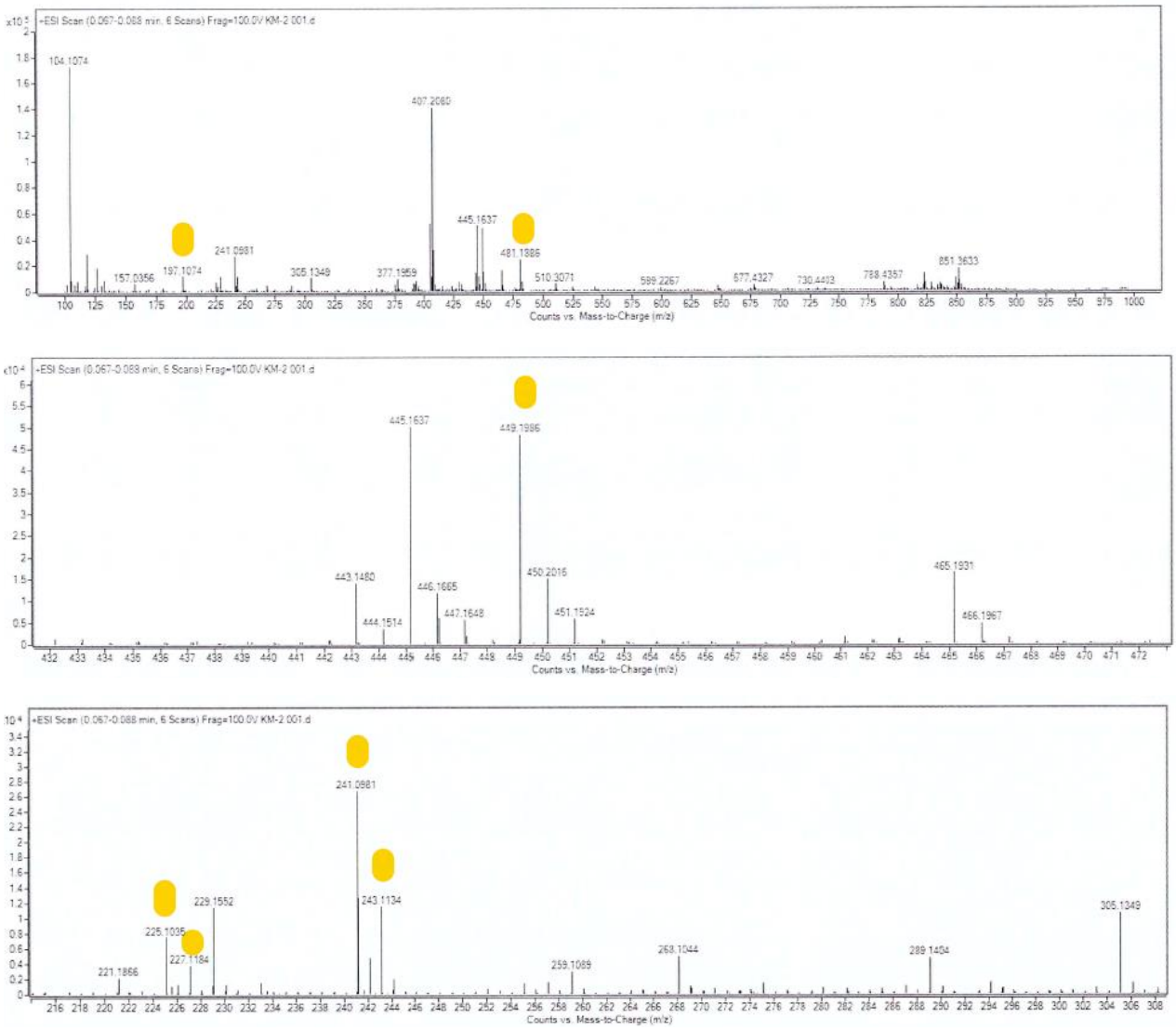

Figure 2. High resolution mass spectra (+ESI) of methanol extract showed the *m/z* $[M+H]^+$ at 197.1074, 225.1035, 227.1184, 241.0981, 243.1134, 449.1986, and 481.1886.

composed of the β-carbolines as same as Indonesian *P. javanica*, shown in Table 3. These compounds are potentially important for development of antimalarial drugs.

Table 3. Comparison of the β-carbolines found in Thai and Indonesian *P. javanica.*

Solvent used	Single extracted	Successively extracted	
	Thai *P. javanica*	**Indonesian *P. javanica***	**Thai *P. javanica***
Chloroform	Dehydrocrenatine, 6-hydroxydehydrocrenatine (Pavanand et al., 1988) crenatine, dehydrocrenatine (Saiin et al., 2016)	1-ethyl-β-carboline, crenatine, picrasidine I, crenatidine, canthin-6-one (Ohmoto et al., 1987)	**Identified by TLC:** (1) 1-ethyl-β-carboline, (2) Crenatine and/or Dehydrocrenatine, (3) 5- or 6- or 8-dehydrocrenatine or 5- and/or 8-hydroxycrenatine, (4) picrasidine T and/or picrasidine G

Table 3. Continues.

Methanol	Picrasidine I (8-hydroxydehydrocrenatine), picrasidine J (8-hydroxycrenatine), picrasidine T (Ohmoto et al., 1987)	**Identified by High resolution MS:** (1) 1-ethyl-β-carboline; m/z 197.1074, (2) Crenatine; m/z 225.1035, (3) Dehydrocrenatine; m/z 227.1184, (4) 5- or 6- or 8- hydroxydehydrocrenatine; m/z 241.0981, (5) 5- or 8-hydroxycrenatine; m/z 243.1134 (6) picrasidine T; m/z 481.1886 / picrasidine G; 449.1986.

CONCLUSION

It could be concluded that *P. javanica* grown in Thailand composed of the β-carbolines similar to that grown in Indonesia. In addition, these results supported the use of *P. javanica* as medicinal plant for treatment of malaria. All fourteen β-carbolines of *P. javanica* should be synthesized and studied their antimalarial activity and toxicity.

ACKNOWLEDGEMENT

This research was financial supported by Faculty of Pharmaceutical Sciences, Naresuan University.

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Citation: Saiin C, Junwised J, Nuengchamnong N, Ingkaninan K, 2019. Determination of β-carbolines in Thai *Picrasma javanica* Bl.; the source of potential antimalarial agents. Adv Med Plant Res, 7(2): 61-67.