SOME CHEMICAL CONSTITUENTS AND SOME BIOLOGICAL ACTIVITIES OF *PICRASMA JAVANICA* FRUIT

Yi Yi Win[1], Yin Yin Htwe[2], Zin Thu Khaing[3], Saw Hla Myint[4]

Abstract

In the present study, two compounds were isolated from the fruit of *Piccrasma javanica* and identified as 1-ethyl-4-methoxy-β-carboline and 1-ethyl-β-carboline on the basis of their spectroscopic data; UV, IR, ^{1}H and ^{13}C NMR in conjunction with 2D experiments (COSY, HSQC, HMBC) and comparison with published literature. For the cytotoxic effect of *P. javanica* fruit ethanol and water extracts; by brine shrimp assay method, the LC_{50} values were found to be 135 µg/mL for the ethanol extract and 318 µg/mL for the water extract. Moreover, the antioxidant potential of ethanol and water extracts was evaluated by DPPH radical scavenging activity, using ascorbic acid as standard and both extracts showed a good antioxidant activity with IC_{50} values of 7.71 µg/mL and 12.19 µg/mL, respectively. Total phenol content by Folin-Ciocalteu reagent method gave 43.06 ± 0.5 mg GAE/g for the ethanol extract and 21.96 ± 1.3 mg GAE/g for the water extract. Furthermore, the anti-inflammatory activity of the ethanol and water extracts by protein denaturation method was 44.44 % and 42.15 %, respectively, at a concentration of 1000 µg/mL.

Keywords: *Picrasma javanica* fruit, cytotoxicity, antioxidant, anti-inflammatory, β-carboline

Introduction

The genus *Picrasma* (Simaroubaceae) comprises about six to nine species native to temperate to tropical regions of Asia and tropical regions of America. The species are shurbs and trees growing up to 20 m high. *Picrasma* species are commonly used in traditional medicine to cure various diseases. They have long been used in herbal medicine as anemopyretic cold, sore throat, dysentery, eczema, nausea, loss of appetite, diabetes mellitus and *falciparum* malaria: the most dangerous type of malaria (Scragg and Allan, 1993). Several alkaloids and quassinoids have been reported from the genus *Picrasma*. *Picrasma javanica* Blume (Synonyms: *Picrasma nepalensis* A.W. Bennet and *Picrasma philippinensis* Elmer.) grows in Java Island at 150-1400 m altitude. The plant occurs from the North-Eastern India throughout South East Asia to the Solomon's islands (Heyne, 1987). The flowers are numerous and whitish. The fruit is a drupe, green, red or blue when it ripens (Figure 1). Traditionally, the plant (also known as Yar-baw-jaw as well as Nann-paw-kyawt in Ka-yin State, Myanmar) is used as a febrifuge where it is known to be a substitute for quinine. Leaves have been applied to festering sore and fruit for inflammation. To date, however, nothing was reported in literature, so far, concerning the chemical composition and biological activities of the fruit of this plant. Therefore, it is deemed worthy of research interest in the fruit of this plant. This paper reports the isolation and identification of two alkaloids from the fruit of *Picrasma javanica*, namely 1-ethyl-4-methoxy-β-carboline (**1**) and 1-ethyl-β-carboline (**2**) and evaluates the cytotoxic effect, and antioxidant and anti-inflammatory activities of the ethanol and water extracts of the fruit.

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(a) (b)

Figure 1 Photographs showing *Picrasma javanica* (a) Plant and (b) Fruit

Materials and Methods

General Experimental Procedure

The FTIR spectra were recorded on a Perkin Elmer GX system FTIR spectrophotometer. UV spectra were recorded on a Shimadzu UV-1800 spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD on JEOL and Varian Inova 400 or 600 MHz spectrometers. The MS spectra were recorded on a Varian MAT 95 Finigan (70eV) ESI-Mass spectrometer. Silica gel 60 (70-230 mesh) Merck was used for column chromatography. TLC analyses were carried out on 0.25 mm silica gel 60 F_{254} precoated on glass plate, Whatman.

Sample Collection

P. javanica fruit used in this study was collected from Naung-ka-mying Township, Ka-yin State (Figure 1). The collected sample was washed with water, and air dried to a constant weight at room temperature for one month. The dried sample was subsequently milled into coarse powder using a grinding mill and then stored in an airtight plastic container for the experimental works.

Preparation of Crude Extracts

The dried fruit powder (300 g) was percolated with ethanol (1000 mL) for 21 days and filtered. This procedure was repeated three times. The combined filtrate was evaporated under reduced pressure by means of a rotatory evaporator to give the ethanol extract. Water extract was prepared by boiling 300 g of dried fruit powder sample in 1000 mL of distilled water for 6 h and filtered and the filtrates were combined followed by heating on water bath and sand bath to give the extract. These extracts were screened for cytotoxic effect, and antioxidant and anti-inflammatory activities.

Isolation of Compounds

The fruit powder of *P. javanica* (1.5 kg) was extracted with EtOH till exhaustion to produce a dark solid extract (60 g), which was then suspended in water (500 mL) and successively partitioned with petroleum ether and ethyl acetate to obtain petroleum ether (1.5g) and ethyl acetate (7.1 g) extracts after removing solvent in vacuum. The ethyl acetate extract was chromatographed on a silica gel column by gradient elution with *n*-hexane–ethyl acetate (100:0–25:75) to obtain six fractions. Then, fraction B was further subjected to silica gel column chromatography by gradient elution with n-hexane: ethyl acetate (80:20–75:25) to give three sub-fractions of B-1 (70 mg), B-2 (80 mg) and B-3 (350 mg). Sub-fraction B-2 was subjected to preparative thin layer chromatography using toluene: chloroform: ethyl acetate (15: 5: 2 v/v) as developing solvent system to yield compound [**1**] (20 mg) and compound [**2**] (5 mg). The structures of the isolated compounds were elucidated and identified by modern spectroscopic techniques, namely UV, FT IR, ^{1}H NMR, ^{13}C NMR, DEPT, and 2D NMR: ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC.

Compound [**1**]: Pale yellow crystals; UV λ_{max} (EtOH) nm: 259, 303, 346; λ_{max} (MeOH/HCl) nm: 248, 303, 367; IR ν_{max} (KBr) cm^{-1}: 3471 (–NH), 3085 (aromatic C-H), 1635(>C=N–), 1593 and 1220 (aromatic benzene and ar-C–O–Me), 1118 (=C–N<) and 2918 and 2854 (–CH_3 and –CH_2–); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 1.

Compound [**2**]: Pale yellow amorphous; UV λ_{max} (MeOH) nm: 242, 285, 348; λ_{max} (MeOH/HCl) nm: 247, 308, 371; IR ν_{max} (KBr) cm^{-1}: 3471 (–NH), 3085 (aromatic C-H), 1635 (>C=N–), 1593 (aromatic benzene and ar-C–O–Me), 1118 (=C–N<) and 2918 and 2854 (–CH_3 and –CH_2–); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) see Table 2.

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay method indicates cytotoxicity as well as a wide range of pharmacological activities e.g., anticancer, anti-viral and pesticidal properties. The experiment was carried out using the method described by McLaughlin with slight modifications (McLaughlin *et al*., 1982). Brine shrimp eggs were obtained from a pet shop. Briefly, *Artemia salina* cysts (brine shrimp eggs, 0.1 g) were allowed to hatch in artificial seawater, containing 3.8 g/L sodium chloride. The larvae (nauplii) were placed in the prepared water for 48 h at 25 °C under constant aeration and illumination to ensure survival and maturity before use. Stock solutions (10 mg/mL) of fruit extracts were prepared in clean test tubes of 10 mL volume to obtain five final concentrations (800, 400, 200, 100, 25 μg/mL). Ten nauplii were collected with the aid of a pipette and added to the serially diluted test solutions. Test was carried out in triplicate. The negative control consisted of ten nauplii per tube in sea water without plant extract while potassium dichromate was used as the positive control. After the 24 h incubation at 25 °C, a magnifying lens was used to count the number of dead larvae. Larvae were considered dead only if they did not move for few seconds after pricking with sharp object during observation and the percentage mortality was calculated as follows.

$$\% \text{ Mortality} = \frac{A}{A + B} \times 100$$

Where, A = number of dead nauplii and B = number of live nauplii

The 50 % mortal concentration (LC_{50} value) was calculated using a linear regressive excel programme.

Investigation of Antioxidant Capacity

(i) Determination of total phenolic content

The total phenolic content of the ethanol and water extracts of *P. javanica* fruit were determined with Folin-Ciocalteu reagent by using the method developed by Harbertson and Spayd (Harbertson and Spayd, 2006). A 0.5 mL of extract sample (0.1 %), 2.5 mL of 1/10 dilution of Folin-Ciocalteu reagent and 2.0 mL of sodium carbonate (7.5 %, w/v) in water were mixed and incubated for 15 min at 45 °C. The reaction was kept in the dark for 30 min and after centrifuging the absorbance of blue color from different samples was measured at 765 nm with a visible spectrophotometer. The phenolic content was calculated as gallic acid milligram equivalents GAE/g of dry plant material on the basis of a standard curve of gallic acid. All determinations were carried out in triplicate.

(ii) Determination of *in vitro* antioxidant action by DPPH free radical scavenging assay

The radical scavenging and antioxidant activity of ethanol and water extracts of *P. javanica* fruit was estimated alongside the free radical DPPH (Brand-Williams *et al.*, 1995). Various

concentrations (2.5, 5, 10, 20 and 40 μg/mL) of the extracts, and commercial antioxidant (ascorbic acid) (1.5 mL) were incubated with a 0.002 % DPPH solution (1.5 mL) for about 30 min at room temperature in the dark. A vortex machine was employed to ensure a thorough mixing and the absorbance was read at 517 nm by UV-Vis spectrophotometer. The control solution was also prepared by mixing 1.5 mL of 0.002 % DPPH and 1.5 mL of EtOH solutions. The capacity of the crude extracts to scavenge DPPH free radicals was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A_{DPPH} - (A_{sample} - A_{blank})}{A_{DPPH}} \times 100$$

Where, A_{DPPH} = absorbance of DPPH in EtOH solution
A_{sample} = absorbance of test sample and DPPH solution
A_{blank} = absorbance of test sample in EtOH solution

The dose-response curve was plotted, and the IC_{50} value of the commercial antioxidant and crude extracts were calculated by using a linear regressive excel programme.

Determination of *In Vitro* Anti-inflammatory Activity by Protein Denaturation Method

Anti-inflammatory activity of ethanol and water extracts of *P. javanica* fruit was evaluated by protein denaturation method (Elias and Rao, 1988). The reaction mixture consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (pH 6.4) and 2 mL of varying concentrations (25, 100, 200, 400, 800 μg/mL) of the test extracts and diclofenac sodium which was used as reference. Similar volume of double-distilled water served as control. Then the mixtures were incubated at 37 °C ± 2 °C in an incubator for 15 min and then heated at 70 °C for 5 min. After cooling, the turbidity was measured at 660 nm with spectrophotometer. The percentage inhibition of protein denaturation was calculated by using the following formula;

$$\% \text{ Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where, $A_{control}$ = absorbance of control

A_{sample} = absorbance of test sample

The 50% inhibition of protein denaturation (IC_{50} value) was calculated using a linear regressive excel programme.

Results and Discussion

Structure Elucidation of Isolated Compounds

The ethyl acetate soluble fraction from ethanol extract of *Picrasma javanica* fruit was subjected to a succession of chromatographic procedures, including silica gel chromatography to afford two pure isolates. The structures of the isolated compounds in Figures 2 and 3 were established from spectroscopic analysis, especially, NMR spectra in conjunction with 2D experiments (COSY, HMQC, and HMBC) and direct comparison with published data. The ^{1}H and ^{13}C NMR data of isolated compound **1** (Figures 7-10) resemble strikingly with those reported for 1-ethyl-4-methoxy-β-carboline (Ohmoto *et. al*., 1987). The IR spectral data also revealed the presence of singlet –NH group (3471 cm^{-1}), aromatic =CH– group (3085 cm^{-1}) group, >C=N– group (1635 cm^{-1}), aromatic benzene and ar-C–O–Me group (1593 cm^{-1} and 1220 cm^{-1}) and =C–N< group (1118 cm^{-1}). In UV spectral data, the absorption maximum for the lowest energy $\pi \rightarrow \pi^*$ electronic transition observed at 346 nm in EtOH shifted 21 nm to a longer wavelength maximum at 367 nm by addition of HCl to the EtOH solution. This indicates a β-carboline structure (Tarzi *et al.*, 2005). The isolated compound **1** must therefore be 1-ethyl-4-methoxy-β-

carboline: DEPT, ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC data of compound **1** also agreed with the structure of 1-ethyl-4-methoxy-β-carboline (Figure 2 and the comparative spectral data in Table 1).

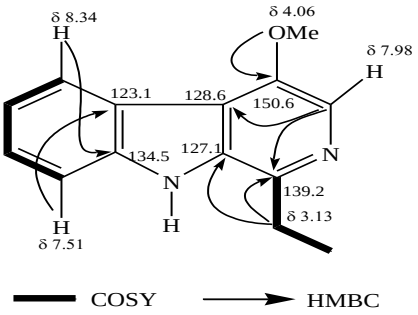

Figure 2 Structure of compound **1** (1-ethyl-4-methoxy-β-carboline)

Table 1 ^{1}D and ^{2}D NMR Spectral Data ($CDCl_3$, 500 and 125 MHz) of Compound 1and Reported Data ($CDCl_3$, 500 and 125 MHz) of 1-ethyl-4-methoxy-β-carboline (J_{Hz} Value in Parenthesis)

Position	C-type (DEPT)	compound 1		1-ethyl-4-methoxy -β-carboline*		^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC
		^{1}H NMR (δ_H/ppm)	^{13}C NMR (δ_C/ppm)	^{1}H NMR (δ_H/ppm)	^{13}CNMR (δ_C/ppm)		
1	C	-	139.2	-	139.2		
2	-	-	-	-	-		
3	=CH–	7.98, s	120.6	8.00, s	120.6		H-3→C-1, C-4b
4	C	-	150.6	-	150.6		
4a	C	-	123.1	-	123.1		
4b	C	-	128.6	-	128.6		
5	=CH–	8.34, d (8.0)	124.5	8.35, d (8.0)	124.5	H-5→H-6	H-5→C-8a
6	=CH–	7.29, m	120.2	7.27, m	120.2	H-6→H-5	
7	=CH–	7.50, t (7.6)	127.6	7.49, m	127.6	H-7→H-8	
8	=CH–	7.51, m	111.2	7.49, m	111.2	H-8→H-7	H-8→C-4a
8a	C	-	134.5	-	134.5		
8b	C	-	127.1	-	127.1		
9	-	-	-	-	-		
1'	CH_2	3.13, q (7.6)	26.5	3.13, q (8)	26.5	H-1′→H-2'	H-1′→C-1, C-8b
2'	CH_3	1.36, t (7.6)	14.4	1.37, t (8)	14.4	H-2′→H-1'	H-2′→C-1
4-OMe	CH_3	4.06, s	56.1	4.07, s			4OMe→C-4

* Ohmoto *et al*., 1987

Compound 2

There is a slight difference from compound **1** in ^{1}H and ^{13}C NMR data of the compound **2** (Figures 11 and 12), the methoxyl group signal at C-4 disappeared and the new aromatic methine signal appeared at δ_H 7.82 ppm and δ_C 112.8 ppm. The aromatic methine proton signal (δ_H 7.82 ppm) was correlated to the neighbouring proton (δ_H 8.42 ppm) in ^{1}H-^{1}H COSY spectrum (Figure 13) and δ_C 112.8 ppm in the HMQC spectrum of compound **2 (**Figure 14) compared to

compound **1** (Table 1). This fact confirms that –OMe group was replaced by an aromatic hydrogen at C-4. The aromatic =CH at C-4 was confirmed by HMBC correlation of H-4 with C-8b (Figure 15). The comparison of ^{1}H and ^{13}C NMR chemical shifts as well as the observed coupling constants with the reported values (Prinsep, 1990) were presented in Table 2. Thus compound **2** was proposed as 1-ethyl-β-carboline (Figure 3).

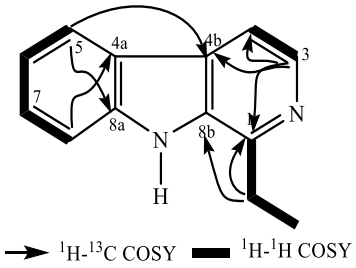

Figure 3 Structure of compound **2** (1-ethyl-β-carboline)

Table 2 ^{1}D and ^{2}D NMR Spectral Data ($CDCl_3$, 500 and 125 MHz) of Compound 1 and Reported Data ($CDCl_3$, 500 and 125 MHz) of 1-ethyl-β-carboline (J_{Hz} Value in Parenthesis)

Position	C– type (DEPT)	Compound 2		1-ethyl-β-carboline*		^{1}H-^{1}H COSY	^{1}H-^{13}C HMBC
		^{1}H NMR (δ_H/ppm)	^{13}CNMR (δ_C/ppm)	^{1}H NMR (δ_H/ppm)	^{13}CNMR (δ_C/ppm)		
1	C	-	146.6	-	146.8	-	-
2	-	-	-	-	-	-	-
3	=CH-	8.42, d (5.1)	138.8	8.43, d (5.3)	138.7	H-3→H-4	H-3→C-1, C-4, C-4b
4	=CH-	7.82, d (5.1)	112.8	7.83, d (5.3)	112.9	H-4→H-3	H-4→C-8b
4a	C	-	122.1	-	122.0	-	-
4b	C	-	128.6	-	128.6	-	-
5	=CH-	8.11, d (8.0)	121.7	8.11, d (8.0)	121.7	H-5→ H-6, H-7	H-5→C-4b, C-8a
6	=CH-	7.28, t (7.6)	120.1	7.27, m	119.9	H-6→H-5	-
7	=CH-	7.52, t (7.6)	128.2	7.49, m	128.1	H-7→ H-5, H-8	-
8	=CH-	7.52, m	111.4	7.49, m	111.5	H-8→H-7	H-8→C-4a
8a	C	-	140.1	-	140.3	-	-
8b	C	-	133.8	-	-	-	-
9	-	-	-	-	-	-	-
1'	CH_2	3.16, q (7.6)	27.2	3.16, q (7.6)	27.2	H-2'	H-1′→C-1, C-8b
2'	CH_3	1.42, t (7.6)	12.6	1.41, t (7.6)	12.6	H-1'	H-2′→C-1

* Prinsep, 1990

Determination of Cytotoxic Effect

The cytotoxicity of ethanol and water extracts of *P. javanica* fruit was determined by using brine shrimp lethality bioassay. The percent mortality of brine shrimp in different concentrations and LC_{50} value of extracts and that of positive control; potassium dichromate was shown (Table 3 and Figure 4). From the results, LC_{50} value of EtOH extract showed 135 μg/mL and that of H_2O extract was 380 μg/mL. It could be deduced that EtOH and H_2O extracts of *P. javanica* fruit had

medium toxicity due to LC_{50} values between 100 and 500 µg/mL. Therefore, the cytotoxic effect of the extracts might be due to the presence of cytotoxic compounds in the fruit extracts.

Table 3 Percent Mortality and LC_{50} Value of Crude Extracts of *P. javanica* Fruit

Extracts	% Mortality of Brine Shrimp ±SD in Different Concentrations of Sample (µg/mL)					LC_{50} (µg/mL)	Toxicity Profile
	25	100	200	400	800		
EtOH	31±0.65	47±0.78	55±0.48	62±1.09	68±1.05	135	Toxic
Water	19±0.75	35±0.77	42±0.49	52±0.33	56±0.62	380	Toxic
*$K_2Cr_2O_7$	42±0.56	61±0.73	72±1.15	80±0.60	88±0.61	48	Highly Toxic

*- positive control and N = 10 (no. of shrimps).
Score for LC_{50}: highly toxic; 0-100 µg/mL, toxic; 100-500 µg/mL, non-toxic; > 1000 µg/mL (Mentor *et al.*, 2014)

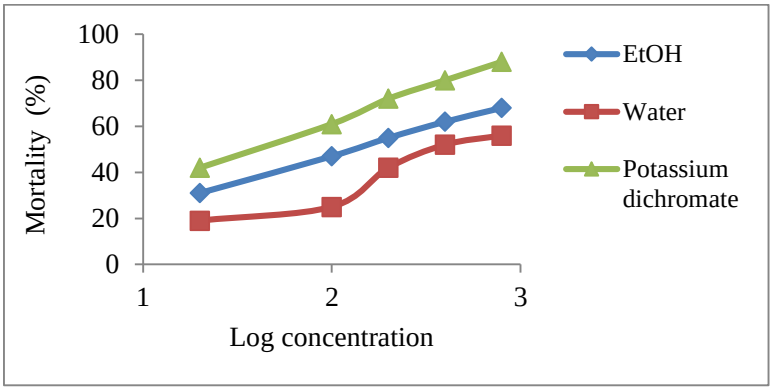

Figure 4 Brine shrimp mortality of crude extracts of *P. javanica* fruit for 24 h

Determination of Total Phenolic Content

The total phenol content of ethanol extract of *P. javanica* fruit (43.06 ± 0.5 mg GAE/g) was found to be higher than water extract (21.96 ± 1.3 mg GAE/g) (Table 4). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds. These correlations indicated that high total phenol contents in ethanol extract contributed to high antioxidant and anti-inflammatory activities of this extract.

Determination of *In Vitro* Antioxidant Effect

In the DPPH free radical scavenging assay, the ethanol extract of *P. javanica* fruit revealed maximum free radical scavenging activity (IC_{50} = 7.71 µg/mL) when compared to ascorbic acid (IC_{50} = 3.87 µg/mL) (Table 4 and Figure 5). This prominent free radical scavenging activity may be due to synergistic activity of various chemical entities present in the extractive.

Table 4 Percent Inhibition of DPPH Free Radicals, IC_{50} Value and Total Phenolic Content of Crude Extracts of *P. javanica* Fruit

Extracts	Inhibition of DPPH Free Radicals (% Mean± SD) at Different Concentrations (μg/mL)					IC_{50} (μg/mL)	Total phenol content (mg GAE/g)
	2.5	5	10	20	40		
EtOH	30.80 ±1.06	41.14 ±0.44	57.63 ±1.56	62.78 ±1.44	71.68 ±0.42	7.71	43.06 ± 0.5
Water	25.17 ±0.16	34.66 ±0.44	44.85 ±1.47	57.91 ±0.79	63.68 ±1.53	12.19	21.96 ± 1.3
Ascorbic acid*	43.81 ±0.88	55.09 ±0.11	67.5 ±0.36	71.71 ±1.06	80.21 ±0.44	3.87	-

Data are expressed as means ± SD from triplicate experiments.
*- positive control

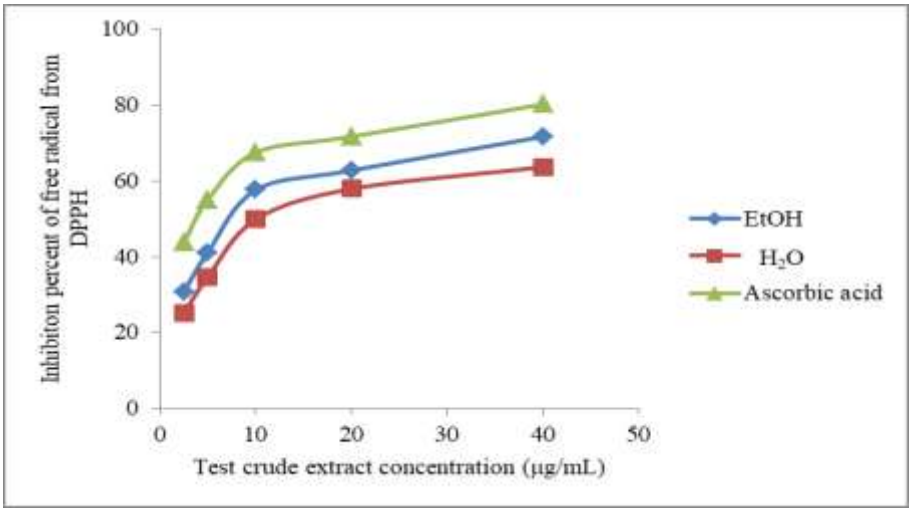

Figure 5 Percent inhibition of DPPH free radicals of crude extracts of *P. javania* fruit tested

Determination of *In Vitro* Anti-inflammatory Effect

Protein denaturation is the process by which proteins lose their tertiary structure and secondary structure. Protein denaturation is a well-documented cause of inflammation. From the results of this study, the ethanol and water extracts of *P. javanica* fruit are effectively inhibiting the protein denaturation (albumin) caused by heat. The protein denaturation percent inhibition was 42.51 % and 44.44 %, respectively, for the ethanol and water extracts at a concentration of 1000 μg/mL, whereas diclofenac sodium had produced 89.19 % inhibition (Table 5 and Figure 6).

Table 5 Inhibition Effect of Test Crude Extracts of *P. javanica* Fruit on Protein Denaturation

Extracts	Inhibition (% Mean± SD) at Different Concentrations (μg/mL)			
	100	200	500	1000
EtOH	29.44±0.11	32.79±1.36	37.30±0.32	44.44± 0.79
Water	27.33± 1.30	29.26±0.88	33.83±0.44	42.51±1.32
Diclofenac sodium*	74.41± 2.11	76.72±2.61	81.48± 1.53	89.19±1.02

Data are expressed as means ± SD from triplicate experiments. *- positive control

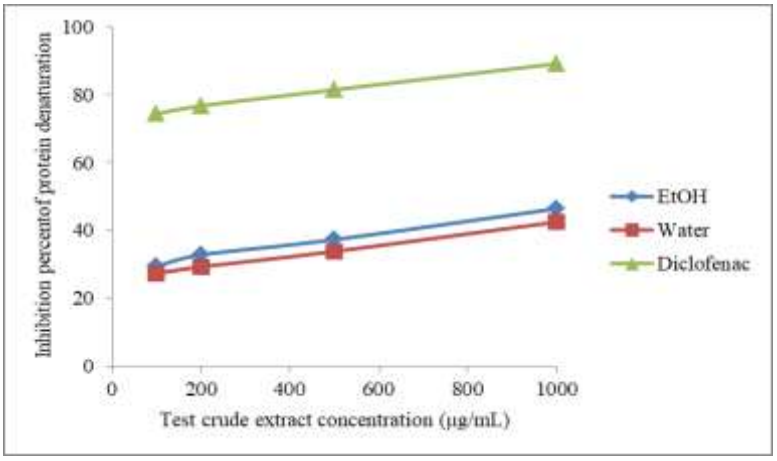

Figure 6 A plot of inhibition percent of protein denaturation against various concentrations of crude extracts of *P. javanica* fruit tested

Analysis Supplementary data for Compound 1

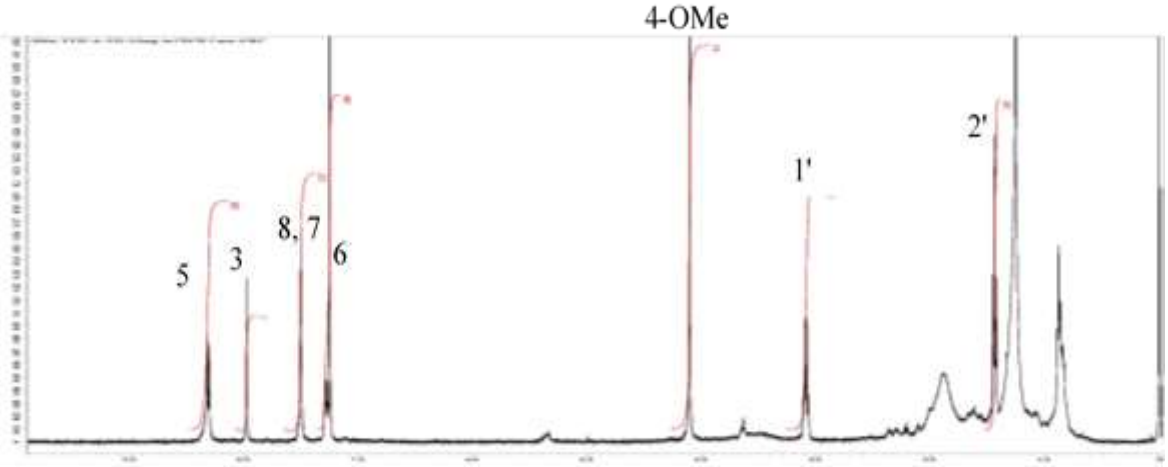

Figure 7 ^{1}HNMR spectrum of compound **1** ($CDCl_3$, 500 MHz)

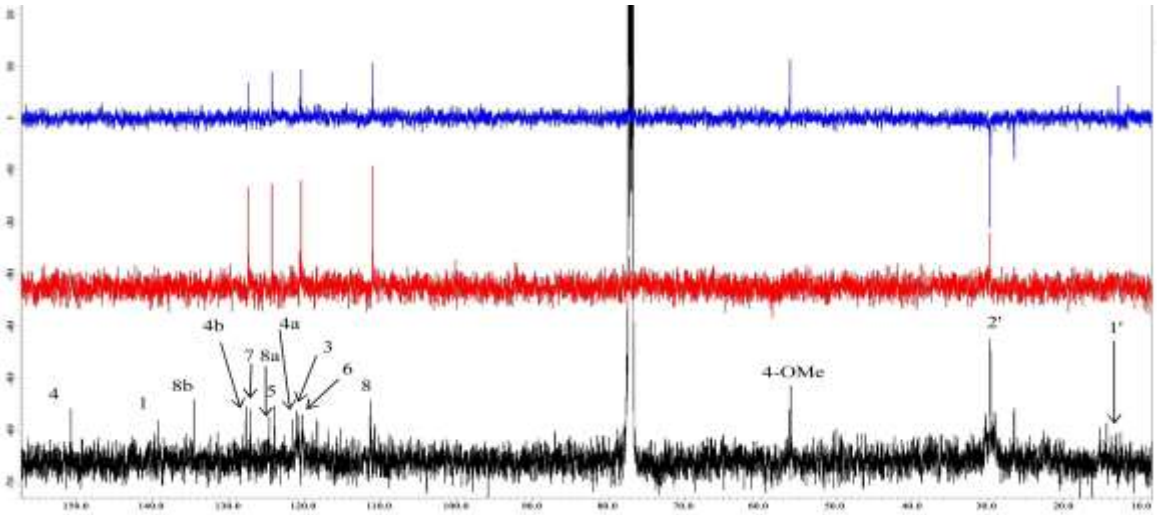

Figure 8 ^{13}C NMR and DEPT spectra of compound **1** ($CDCl_3$, 125 MHz)

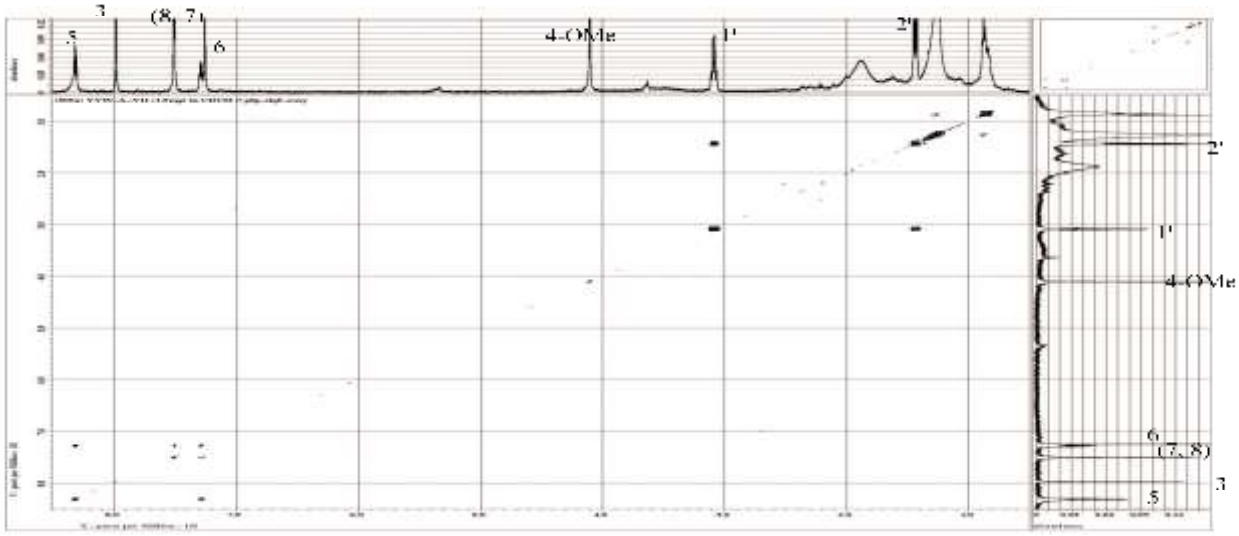

Figure 9 ^{1}H-^{1}HCOSY spectrum of compound **1** ($CDCl_3$, 500 MHz)

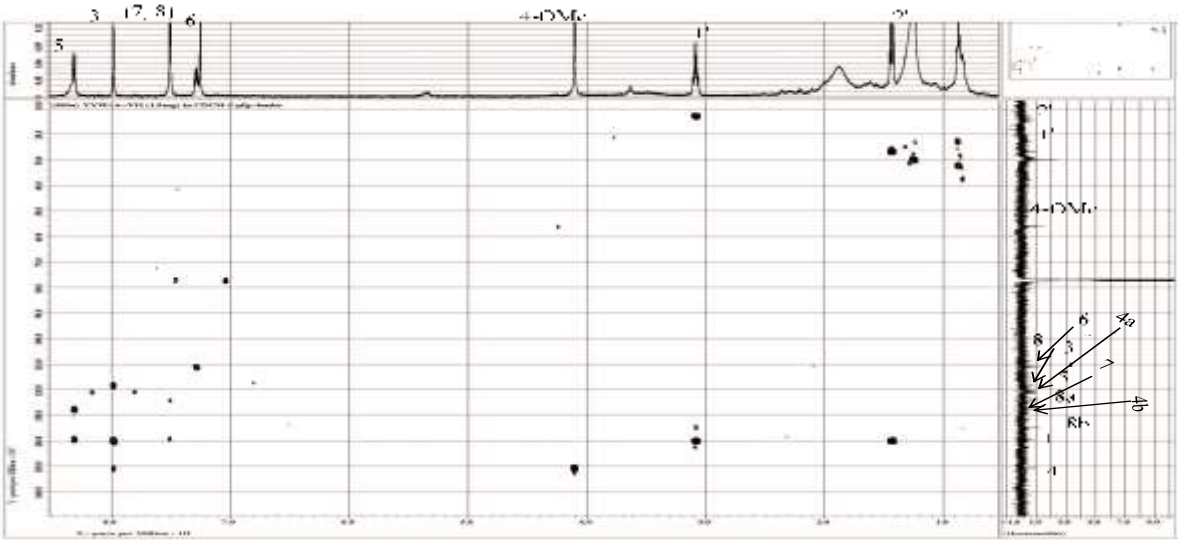

Figure 10 ^{1}H-^{13}C HMBC spectrum of compound **1** ($CDCl_3$, 500 MHz)

Compound 2

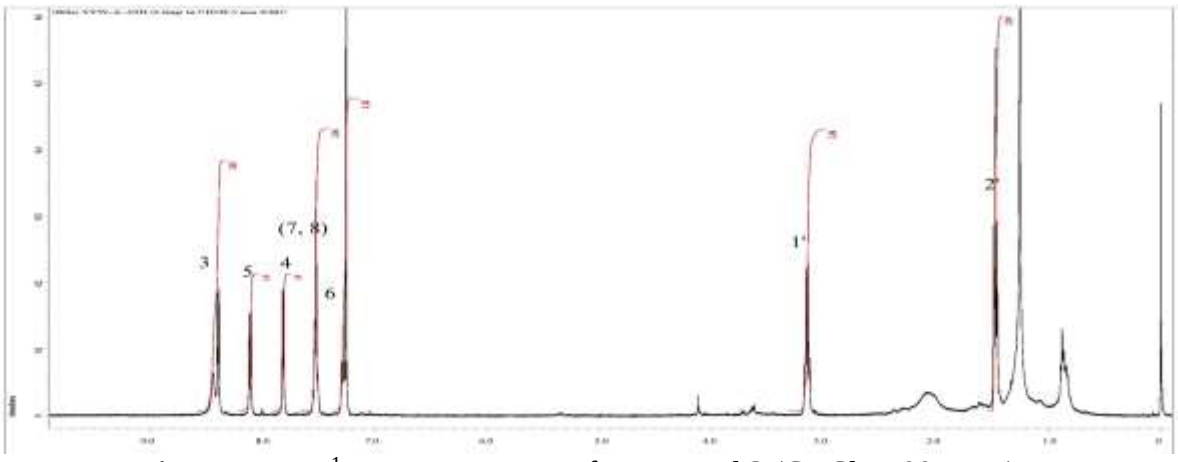

Figure 11 ^{1}HNMR spectrum of compound **2** ($CDCl_3$, 500 MHz)

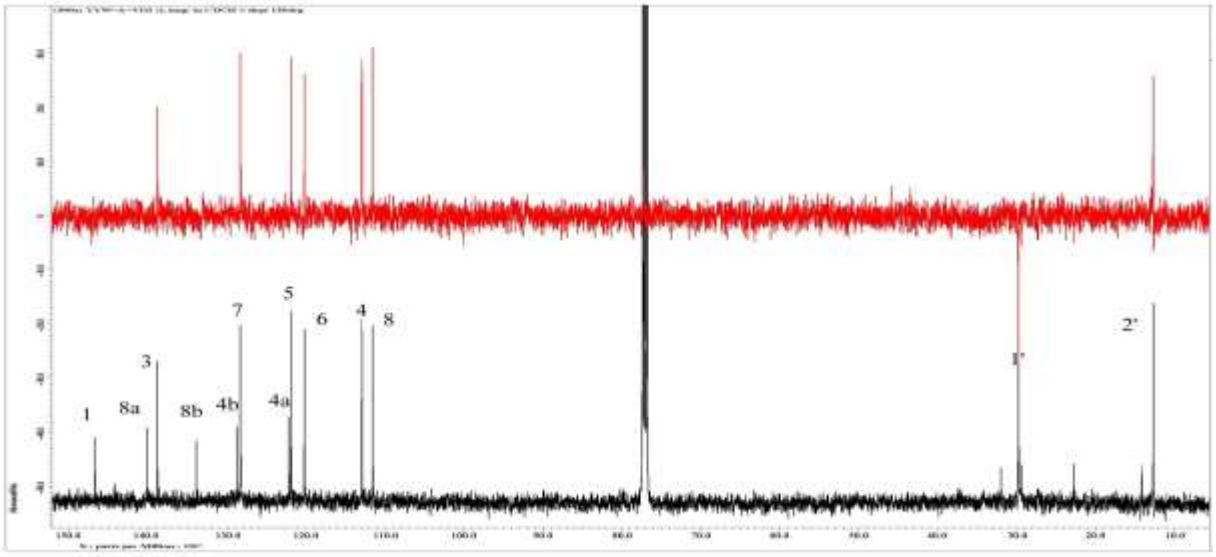

Figure 12 ^{13}C NMR and DEPT spectra of compound **2** ($CDCl_3$, 125 MHz)

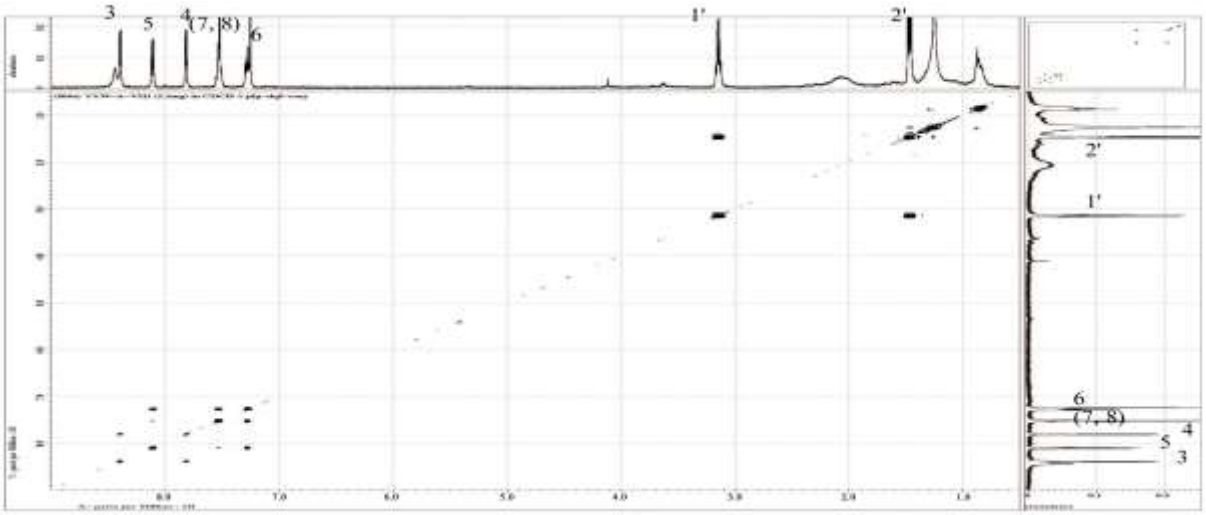

Figure 13 ^{1}H-^{1}H COSY spectrum of isolated compound **2** ($CDCl_3$, 500 MHz)

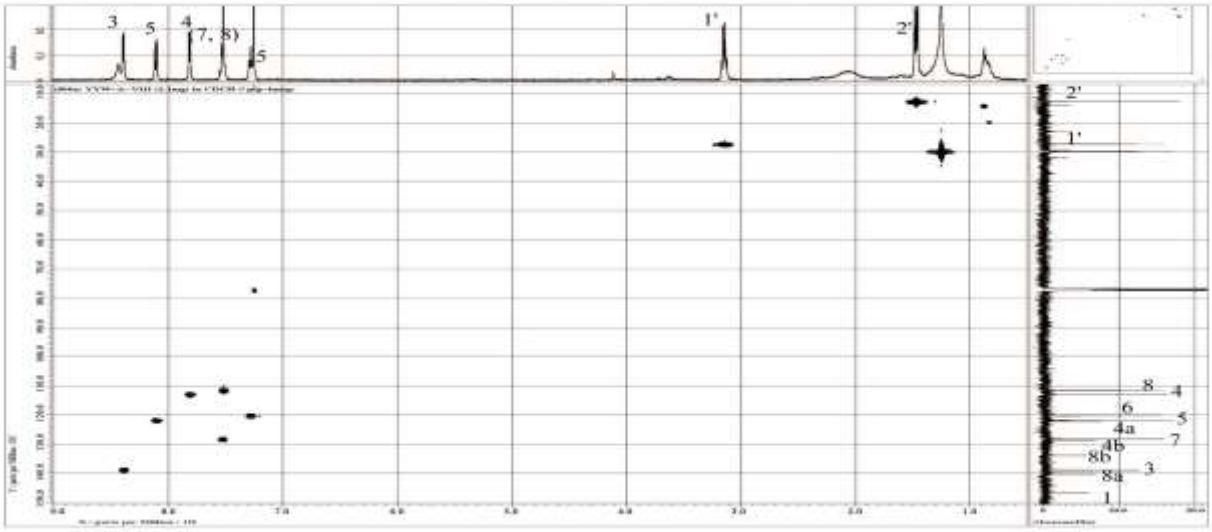

Figure 14 ^{1}H-^{13}C HMQC spectrum of compound **2** ($CDCl_3$, 500 MHz)

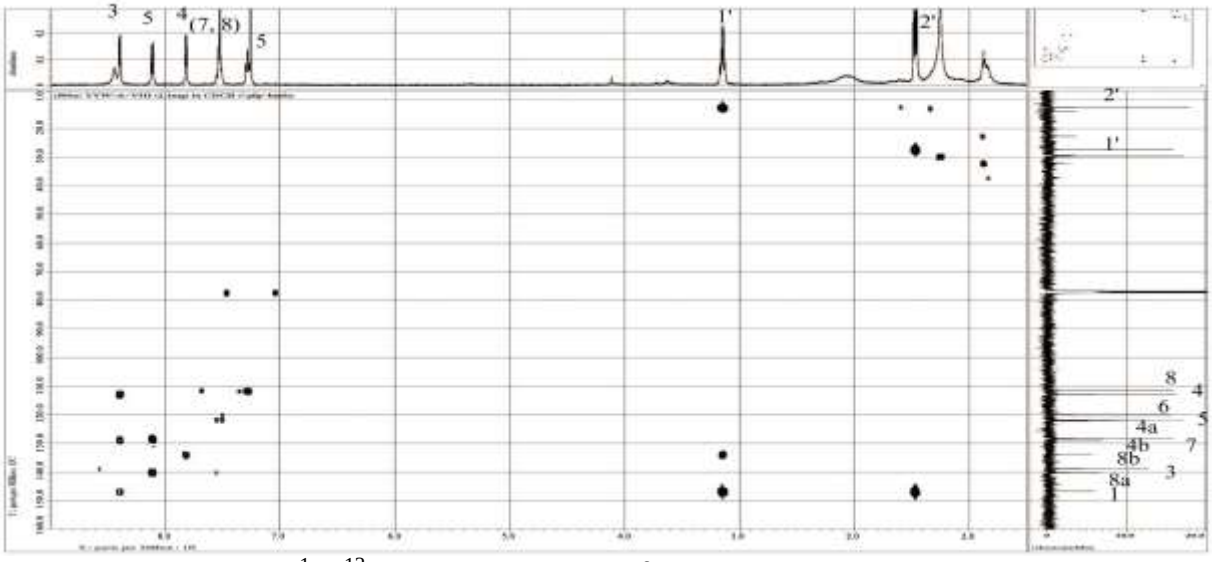

Figure 15 ^{1}H-^{13}C HMBC spectrum of compound **2** ($CDCl_3$, 500 MHz)

Conclusion

This research revealed some chemical constituents such as alkaloids; 1-ethyl-4-methoxy-β-carboline (**1**) and 1-ethyl-β-carboline (**2**), cytotoxicity, antioxidant and anti-inflammatory activities of the *P. javanica* fruit. In fact, the present research showed that the ethanol and water extracts of *P. javanica* fruit possess not only antioxidant but also anti-inflammatory effects as well as cytotoxicity. Therefore, the research findings will contribute to some extent to the search for the *in vivo* antioxidant and anti-inflammatory agents of plant origin and also to the development of the role of Myanmar traditional medicinal formulation, especially in the treatment of the related diseases.

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