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Original Article

Alkaloid from *Phoebe declinata* Nees Leaves

Berna Elya[1,2*], Basah Katrin[1], Roshamur Cahyan Forestrania[1], Rosmalena Sofyan[3] and Ryan Adi Chandra[3]

ABSTRACT

Introduction: Genus Phoebe have been reported to produce isoquinoline alkaloids as aporphines, noraporphines, and benzylisoquinolines. Many of these isolates exhibit diversified biological activities, including cytotoxic activity. **Objective:** The objective of this study is to determine cytotoxic activity of compound isolated from *Phoebe declinata* againts MCF-7 (breast cancer cell line). **Methods:** Extraction was done by reflux using n-hexane, antioxidant activity measured by DPPH method and reducing power method, cytotoxic activity measured by MTT assay using MCF-7 cell line, struture eucidation was confirmed by NMR. **Results**: The antioxidant activity measured using DPPH method for 1 and 2 showed IC_{50} value of 6.42 and 11.80 μg/mL respectively and using reducing power method for 1 and 2 showed IC_{50} value of 7.02 and 13.74 μg/mL respectively. Compound (1) and (2) exhibited cytotoxic activity against MCF-7 cells with an IC_{50} value of 82.978 and 93.179 μg/mL. **Conclusion**: Compound (1) and (2) exhibited antioxidant activity and cytotoxic activity against MCF-7.

Key words: *Phoebe declinata* nees, Alkaloid, Antioxidant activity, DPPH, Cytotoxic activity, MCF-7 cell line.

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INTRODUCTION

Phoebe declinata Nees belongs to Lauraceae family which commonly grows in Indonesia.[1] The plant is a multy years plant (perennial) of moderate size (about 30-40 feet). This plant is called in Indonesia as *huruhejo* or *bedagai*, and grows commonly at Sumatera and Java.[1,2] Genus Phoebe have been reported to produce isoquinoline alkaloids as aporphines, noraporphines, and benzylisoquinolines.[3-5] Many of these isolates exhibit diversified biological activities, including anti-diabetes, anti-inflammation, cytotoxic, antibacterial, antifungal activities and antioxidant properties.[3-6,7] Previous paper, we reported the isolation of alkaloid declinine from stem bark of *Phoebe declinata*.[8] In our present research, a new alkaloid declinatine (1) was obtained from the hexane extract of the plant and a known alkaloid declinine (2) from diclormetana extract (Figure 1).

MATERIALS AND METHODS

General

The ^{1}H-NMR and ^{13}C-NMR were recorded in deuterated chloroform on JEOL 500 MHz instrument. Silica gel 60, 70-230 mesh ASTM (Merck 7734) was used for column chromatography, Mayer's reagent was used for alkaloid screening, TLC aluminum sheets (20 × 20 cm Silica gel 60 F_{254}), were used in the TLC analysis. The TLC spots were visualized under UV light (254 and 366 nm) followed by spraying with Dragenderff's reagent for an alkaloid detection.

Plant Material

The leaves of *Phoebe declinata* (Lauraceae) collected from Bogor, west Java, Indonesia in June 2012, was Identified by Dr. Joko Ridho Witono. A voucher specimen (PD 1065) has been deposited in the Faculty of Pharmacy, University of Indonesia.

Extraction and Isolation

The air-dried leaves *P. declinata* (500g) were reflux in hexane. The plant residue was moistened with 54% of NH_4OH, and exhaustively extracted with dichloromethane by reflux again. The residue was continue extracted with methanol. The hexane, CH_2CL_2 and methanol extracts were evaporated. The hexane extracts (10 g) were subjected to column chromatography using silica gel as stationary phase and n-hexane-ethyl acetate and ethyl acetate-methanol systems, gradually polarity affording 15 fractions. The seven fractions were chromatographed using silica gel and purified to give 1 (40 mg). The dichloromethane extracts (10 g) were subjected to column chromatography using silica gel as stationary phase and ethyl acetate-methanol systems, gradually polarity affording 10 fractions. Fraction 4 was chromatographed using silica gel and purified to give 2 (20 mg).

Free radical scavenging ability using DPPH radical

The antioxidant activity of isolate was assessed by measuring their scavenging potency against stable free radical 1,1 Diphenyl -2-picryl-hydrazyl

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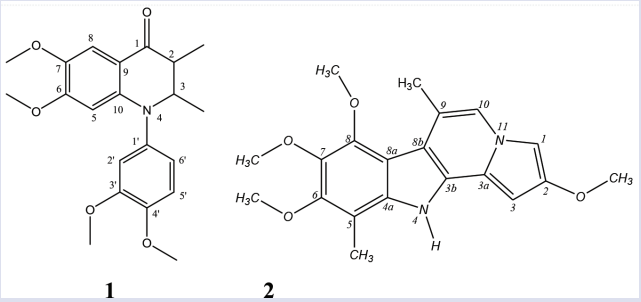

Figure 1: Isolated compounds from leaves of *Phoebe declinata*.

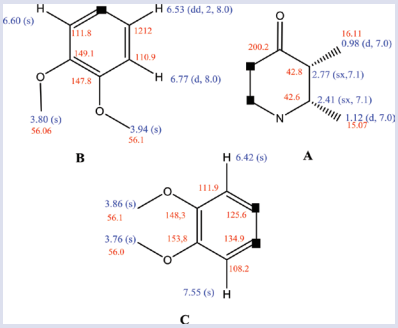

Figure 2: Partial Structures of A, B and C and 1H, 13C-Chemical shift data of Compound 1.

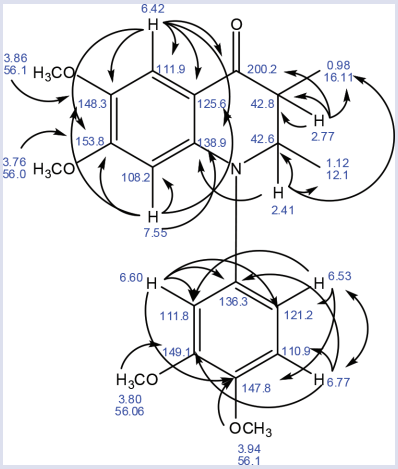

Figure 3: Selected HMBC correlation of Compound 1.

(DPPH).[9] A total of 1 mL of DPPH (100 μg/mL/ solution and 1 mL sample at various concentrations (20, 40, 60 and 80 μg/mL or boldine as the alkaloid standard solution (5,6,7,8,9 and 10 μg/mL were added into mixed solution at the separated place. The reaction mixture was incubation the dark at temperature 37°C for 30 min. Optical density of each solution was measured at 517 nm using methanol as blank. DPPH scavenging activity of samples represented as value of inhibition concentration 50 % was calculated using the following equation:

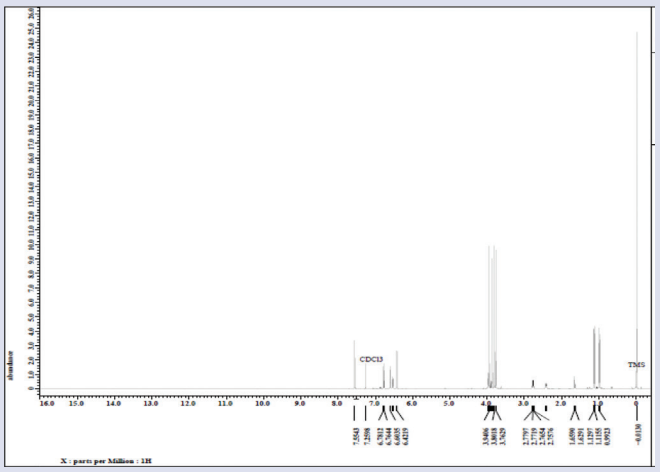

Figure S1: ^{1}H-NMR spectrum compound 1 in $CDCl_3$.

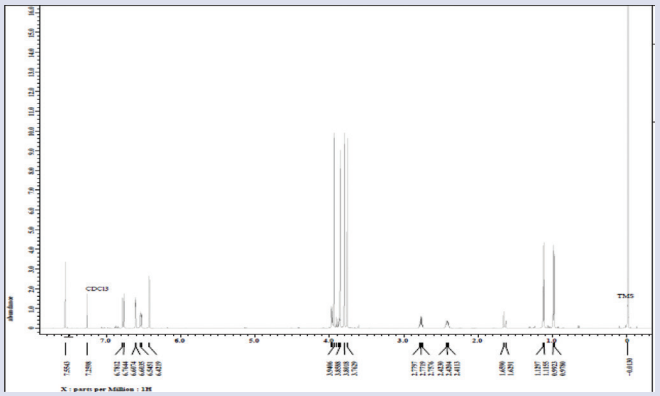

Figure S2: ^{1}H-NMR spectrum compound 1 in $CDCl_3$ (Expanded).

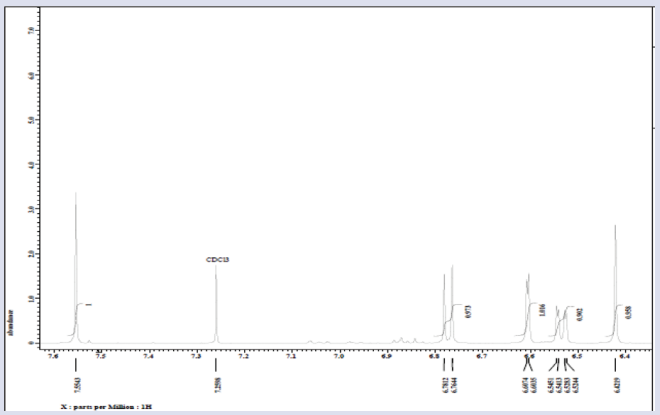

Figure S3: ^{1}H-NMR spectrum compound 1 in $CDCl_3$ (Expanded).

$$(\%)\ \text{activity scavenging} = \frac{\text{A blank} - \text{A sample}}{\text{A blank}} \times 100$$

Free radical scavenging ability using reducing power

The reducing power of the isolate was determined by the method described by Chang *et al*. Different concentrations of the extracts (0.06-1 mg/mL) were mixed with phosphate buffer (0.2 mM, pH 6.5), ferric chloride solution (2 mM) and potassium ferricyanide (4 mM). To this, 100 mg/mL trichloroacetic acid was added to the reaction mixture and was made up to 1 mL with water and incubated at 37°C for 10 min. The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Assay of Cytotoxic activity

The MCF-7 cell line was cultures stock in DMEM with 10 % FBS, 100 μg/mL streptomycin and penicillin (100 IU/mL) and 2 mm glutamine. Cell were incubated in humidified atmosphere of 5% CO_2 at 37°C. 100 μL cell suspension with 1.5 X 10^4 cells included in microplate 96 well. The Samples with concentration 3.125; 6.25; 12.5; 25, 50 and 100 μg/mL with triple replications each cell controls and medium controls. Microplate incubated for 24h at 37°C 2% CO_2, the culture medium removed and washed with PBS. Into each well plate added 10 μL of MTT solution (1 mL MTT in 10 mL culture medium) and microplate incubated at 37°C 2% CO_2. After 4h of stopper reagent added 100 mL of 10 % SDS in 0.1 N HCL into each well (to dissolve the purple formazan crystals). Absorbance is read using an ELISA reader at wavelength of 550 nm.[10,11] The percentage of cell viability and cell death of samples on MCF-7 cell line was calculated for each assay by using the formula:

$$\%\ \text{viability cell} = \frac{\text{ODs} - \text{ODm}}{\text{ODc} - \text{ODm}} \times 100\%$$

*Where ODc = optical density cell with samples, ODc = optical density cell without sample, ODm = optical density media without cell.

Graph percentage of viability cell against logarithm concentration was plottes. The IC_{50} value were calculates by using curve in linier equations.

RESULTS AND DISCUSSION

Compound 1 was obtained as a white crystal. The LCMS-IT-TOF revealed a pseudo molecular ion peak at *m/z* 372.4417 $[M+H]^+$, thus suggesting a molecular weight and formulae are 371.449 and $C_{22}H_{28}O_5$. (calc. 372.45). The ^{1}H-NMR spectrum (Figure S1-S5) contained the presence of three protons of phenyl as ABX type (C ring) at at δ 6.60 (H-2′, *s*), 6.77 (H-5′, *d*, 8.0 Hz) and 6.53 (H-6′, *dd*, 2, 8.0 Hz). Two singlet aromatic protons indicated this signal have para position (B ring), appear at δ_H 6.42 (H-8, *s*) and 7.55 (H-5, *s*). The other signals indicated the presence of ring A : 2 methyls (d) were shown at δ 0.98 (2-Me, *d*, 7.0 Hz) and 1.12 (3-Me, *d*, 7.0 Hz). The presence of two methine at δ 2.77 (sixtet) and 2.41 (sixtet) with 7.1 Hz constants *J* coupling, indicated this signals have cis orientation. Two singlet aromatic protons at δ 6.42 (H-8, s) and 7.55 (H-5, s), indicated this signal have para position in the ring B. Two signal methoxy (-OCH_3) were showed at δ 3.76 (6-OMe, *s*) and 3.86 (7-OMe, *s*). That were ilustrated in partial structure A, B and C, was shown in Figure 2.

The ^{13}C-NMR (Figure S6-S9) and HMQC spectrum (Figure S10) also supported the presence of A, B and C, with the presence of ring C signals at δ 111.8 (C-2′), 110.9 (C-5′) and 121.2 (C-6′). Signals at δ 200.2 (C-1), 42.8 (C-2), 42.6 (C-3,), 16.11 (2-Me) and 12.10 (3-Me) support for the presence of ring A, and signals at 111.9 (C-8), 108.2 (C-5), 56.0 (6-OMe) and 56.1 (7-OMe) confirm for the presence of ring B. Base on this spectral data, indicated that the structure is alkaloid. The compound 1 also showed alkaloid spot which was visualized by Dragendorf's spray method in aluminum sheet. For construct this partial structure was elucidated by use HMBC (Figure S11-S21). The presence of long range coupling in the HMBC experiment between C-2 (δ, 42.6, *d*) and H-3 at δ 4.41 (*d*) and C-10 (δ 133.84, *s*), C-2 (δ 46.04, *d*) indicated ring B was connected with ring A at C-9 and C-10. For construct this partial structure was elucidated by use HMBC experiments (Figure S22).

The presence H-H correlation (COSY) (Figure S7) between H-2 dan H-3 indicated that protons is very close, and the presence of NOESY correlation between H-2 dan H-3, Constant coupling value between H-2 dan H-3 is 7.1 Hz, showed that H-2 is cis to H-2.

Compound 2 was obtained white crystal, m.p. 102-104°C, molecular formula $C_{20}H_{22}N_2O_4$. ^{1}H-NMR ($CDCl_3$, δ): 6.88 (*s*, CH-1), 7.07 (*s*, CH-3), 6.99 (*s*, CH-10), 3,86 (*s*, OCH_3-2), 1.08 (*m*, CH_3-5), 3.88 (*s*, OCH_3-6), 3.89 (*s*, OCH_3-7), 3.9 (*s*, OCH_3-8), 0.67 (*m*, CH_3-9) (Figure S23). ^{13}C-NMR ($CDCl_3$, δ): 118.51 (C-1), 148.99 (C-2), 110.38 (C-3), 147.90 (C-3a), 135,69 (C-3b), 147.78 (C-4a), 133.48 (C-5), 148.60 (C-6), 148,96 (C-7), 148,64 (C-8), 134.84 (C-8a), 133.26 (C-8b), 133.82 (C-9), 109.35 (C-10), 55.88 (OCH_3-2), 11.89 (CH_3-5), 55.87 (OCH_3-6), 55.95 (OCH_3-7), 55.90 (OCH_3-8), 15.05 (CH_3-9) (Figure S24).

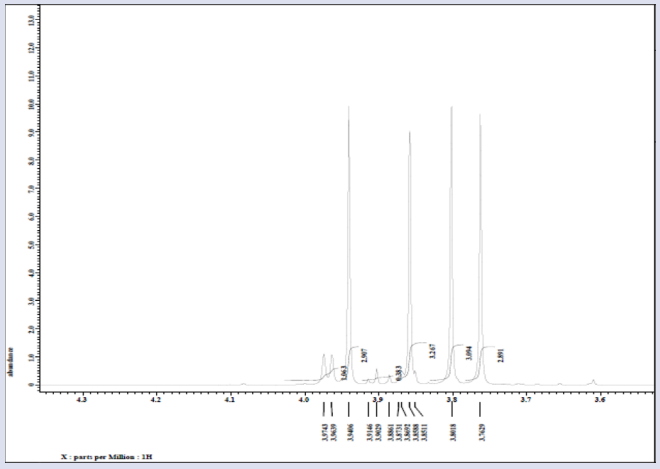

Figure S4: ^{1}H-NMR spectrum compound 1 in $CDCl_3$ (Expanded).

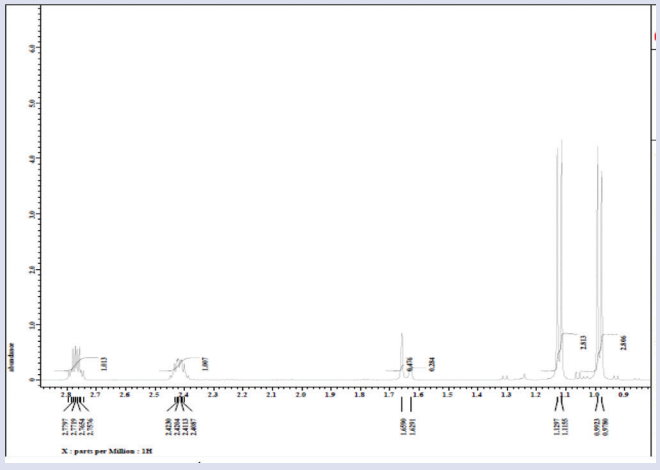

Figure S5: ^{1}H-NMR spectrum compound 1 in $CDCl_3$ (Expanded).

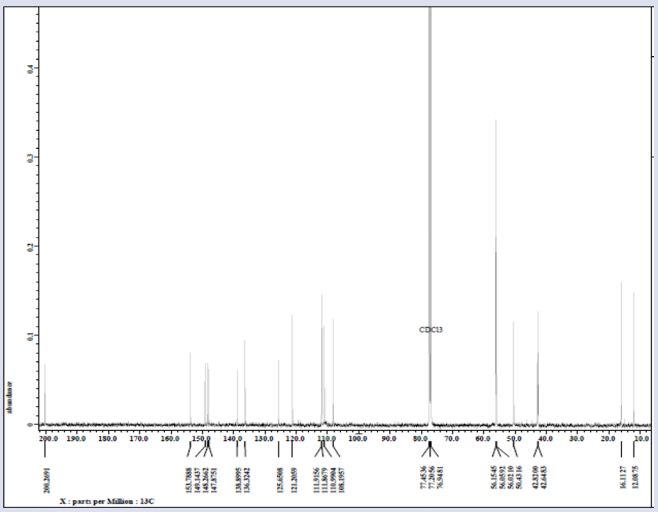

Figure S6: ^{13}C-NMR spectrum compound 1 in $CDCl_3$.

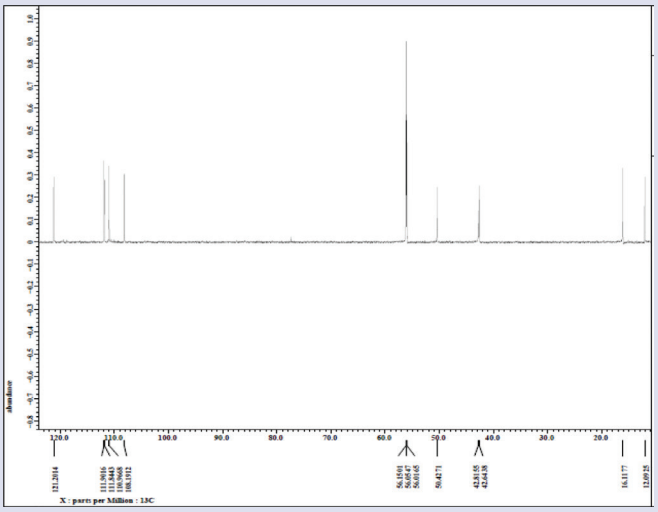

Figure S9: DEPT spectrum compound 1 in $CDCl_3$.

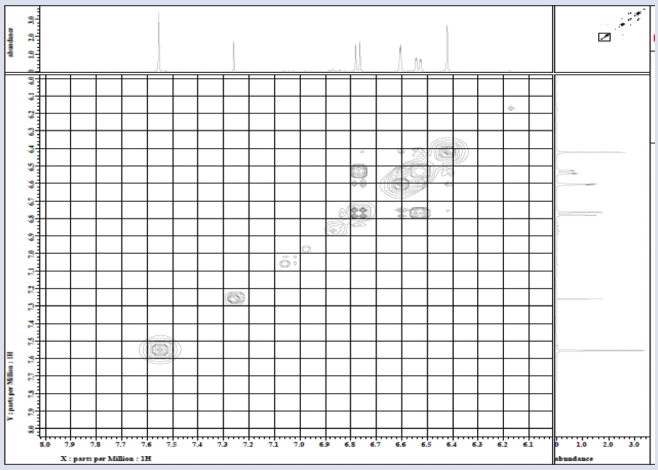

Figure S7: H-H COSY spectrum compound 1 in $CDCl_3$.

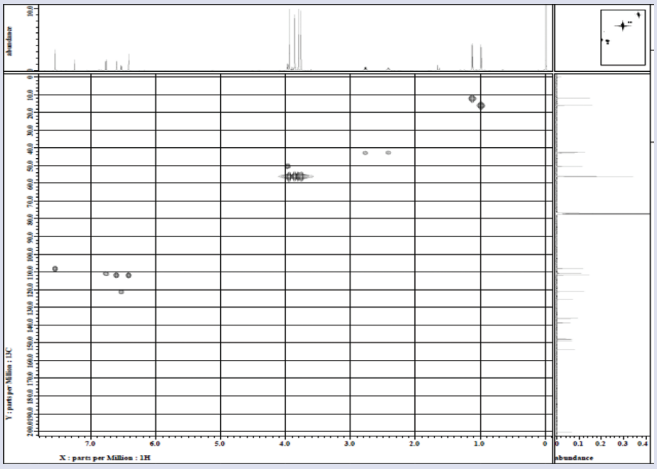

Figure S10: HMQC spectrum compound 1 in $CDCl_3$.

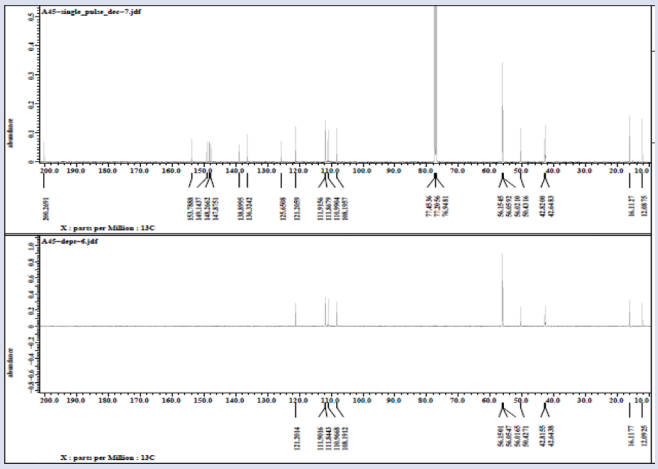

Figure S8: Data slate spectrum compound 1 in $CDCl_3$.

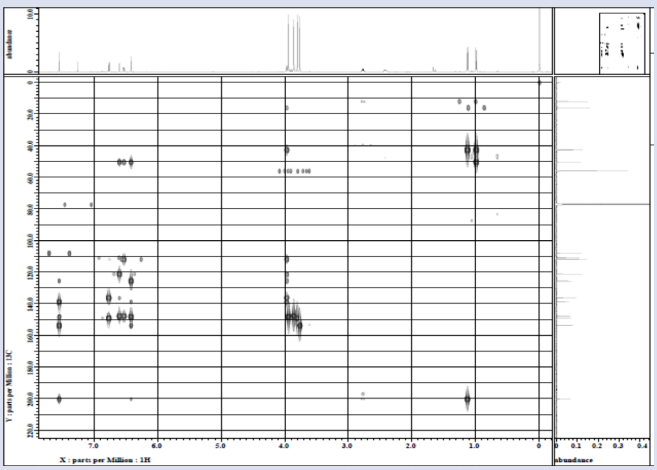

Figure S11: HMBC spectrum compound 1 in $CDCl_3$.

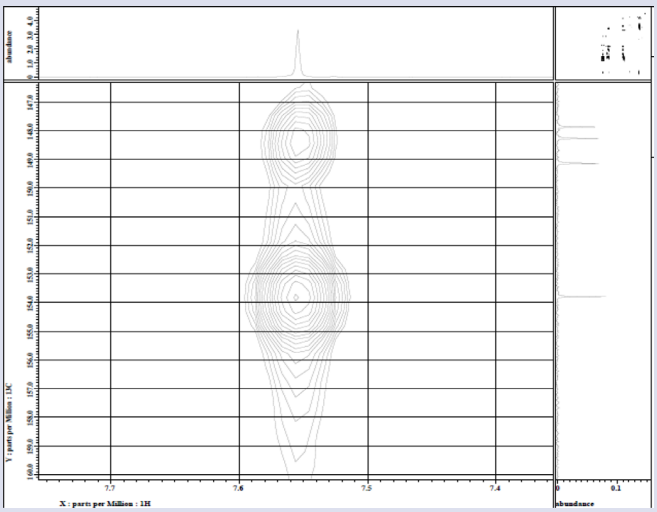

Figure S12: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

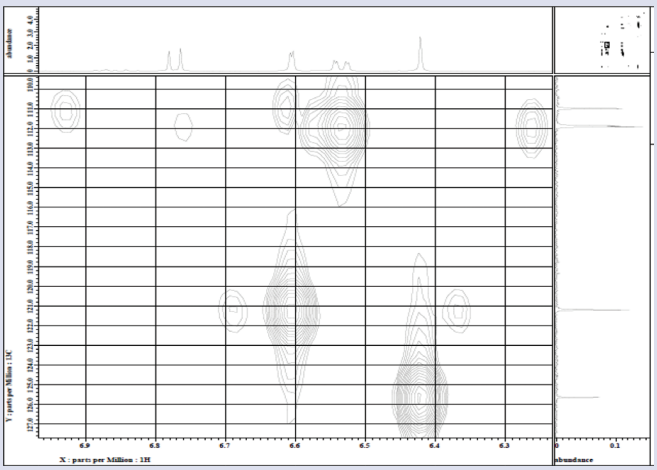

Figure S13: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

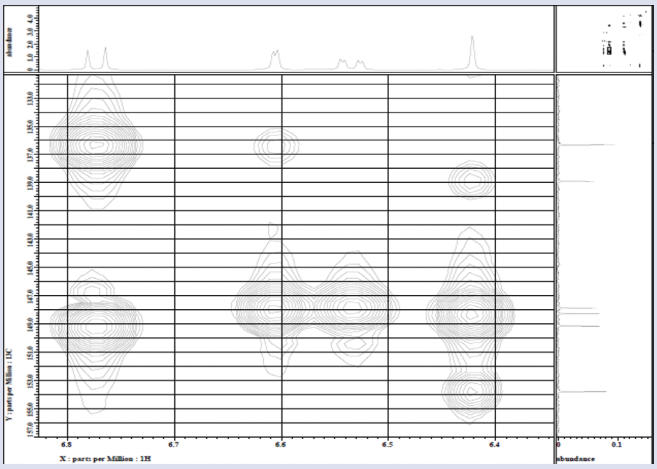

Figure S14: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

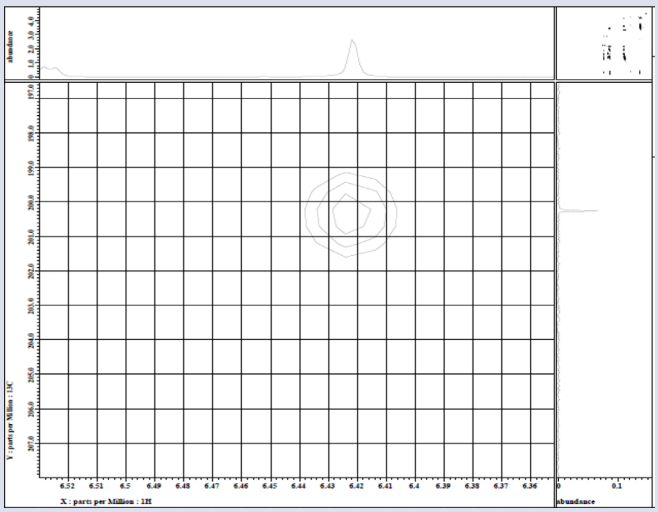

Figure S15: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

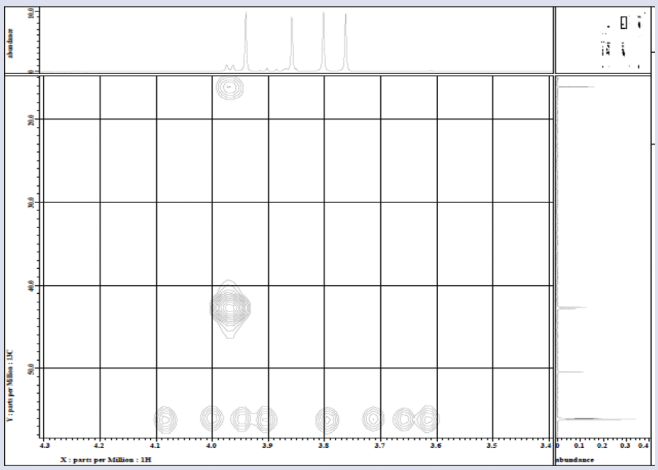

Figure S16: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

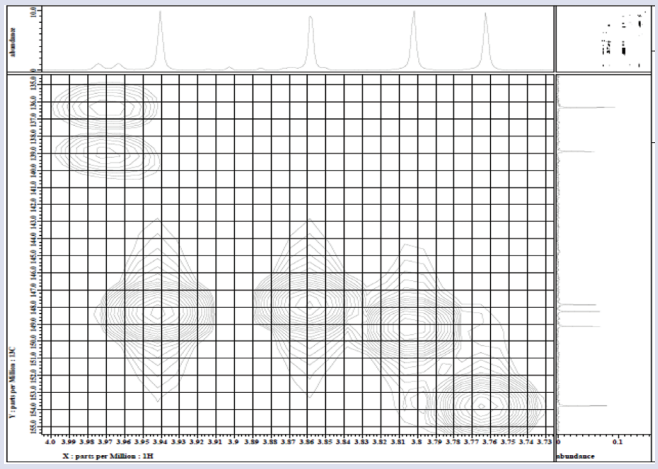

Figure S17: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

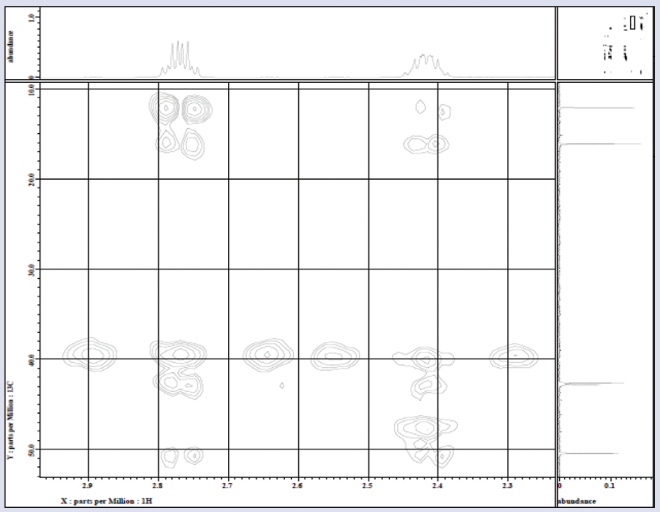

Figure S18: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

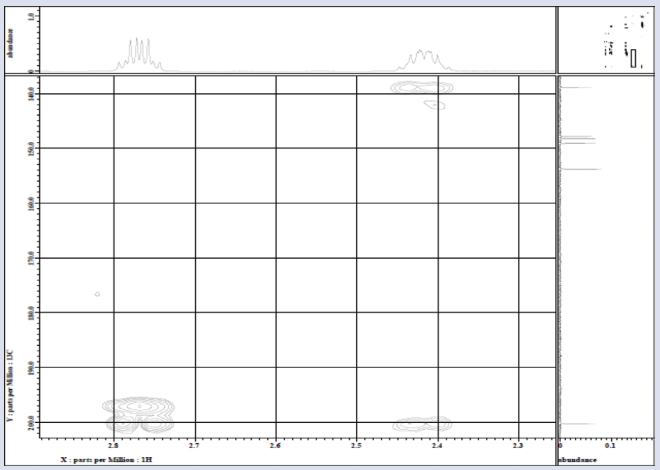

Figure S19: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

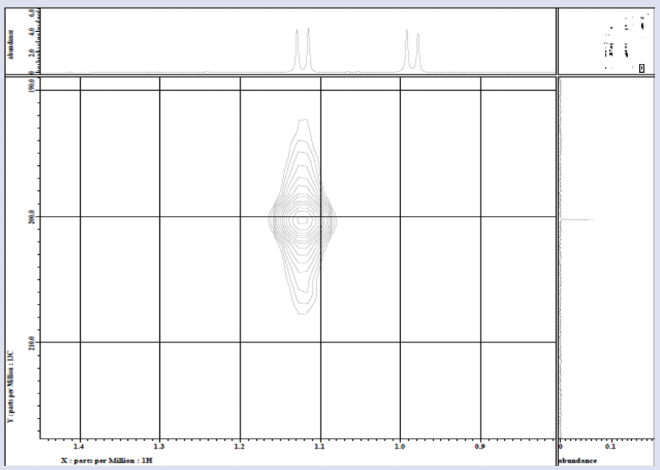

Figure S20: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

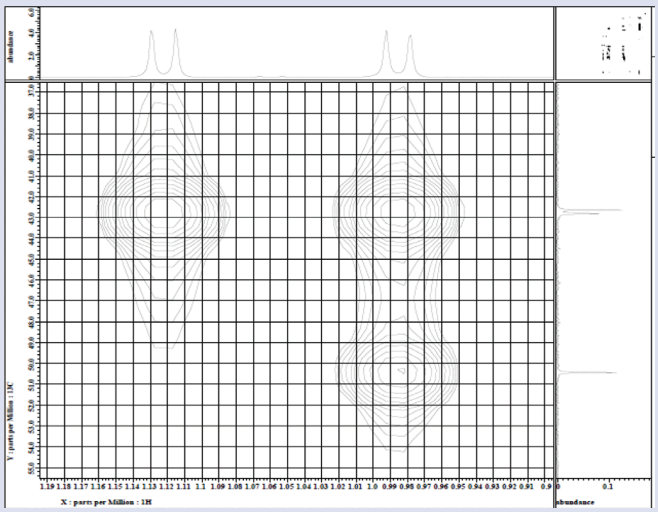

Figure S21: HMBC spectrum compound 1 in $CDCl_3$ (Expanded).

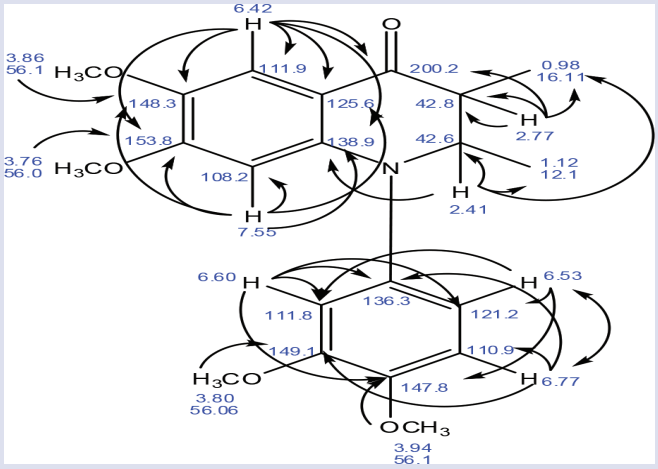

Figure S22: Selected HMBC correlation of Compund 1.

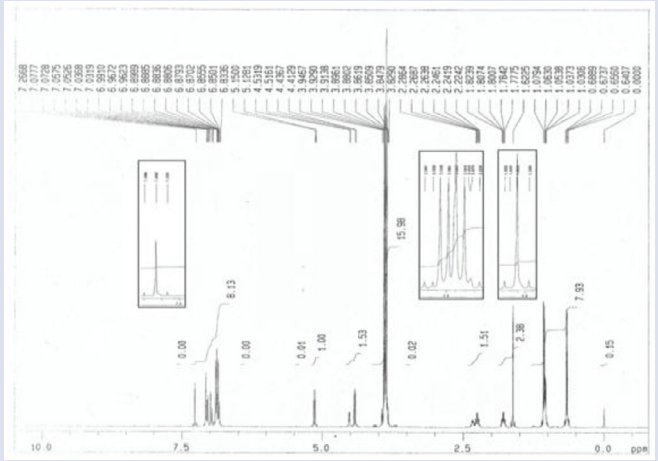

Figure S23: ^{1}H-NMR spectrum compound 2 in $CDCl_3$.

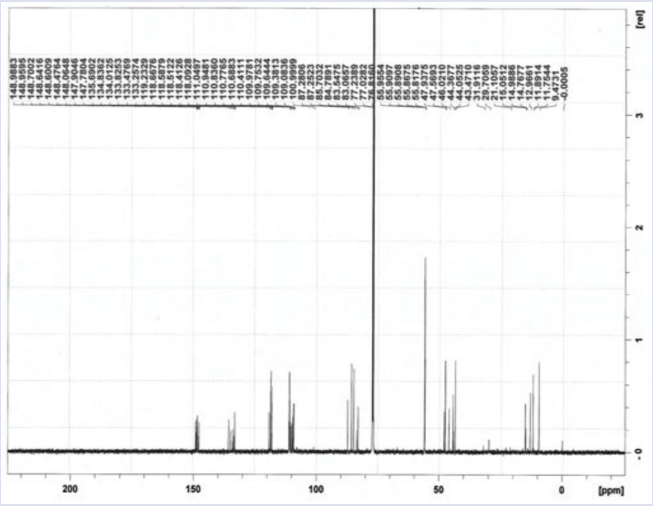

Figure S24: ^{13}C-NMR spectrum compound 2 in $CDCl_3$.

Table 1: ^{1}H-NMR and ^{13}C-NMR assignment for compound 1 in $CDCl_3$.

No	δ_H	δ_C
1	-	200.2
2	2.77 (sektet, 7.1)	42.8
2-CH_3	0.98 (d, 7.0)	16.11
3	2.41 (sektet, 7.1)	42.6
3-CH_3	1.12 (d, 7.0)	12.10
5	7.55 (s)	108.2
6	-	153.8
6-OCH_3	3.76 (s)	56.0
7	-	148.3
7-OCH_3	3.86 (s)	56.1
8	6.42 (s)	111.9
9	-	125.6
10	-	138.9
1′	-	136.3
2′	6.60 (s)	111.8
3′	-	149.1
3′-OCH_3	3.80 (s)	56.06
4′	-	147.8
4′-OCH_3	3.94 (s)	56.1
5′	6.77 (d, 8)	110.9
6′	6.53 (dd, 2; 8)	121.2

Table 2: Result of Antioxidant Activity and Cytotoxic Activity

Sample Name	Antioxidant activity (µg /mL)		Cytotoxic activity(µg /mL)
	DPPH Method	Reducing Power Assay	
Compound 1	6.42	7.02	82.978
Compound 2	11.80	13.74	93.179

Compound 1 and 2 were considered as good antioxidant agent with IC_{50} value 6.42 and 11.80 μg/mL respectively which is compared to boldine as alkaloid standard with IC_{50} 5.80 μg/mL by DPPH methode and by reducing power assay for 1 and 2 with IC_{50} value 7.02 and 13.74 μg/mL respectively which is compared to boldine with IC_{50} 5.95 μg/mL. Table 1. Based on the result of Table 2 shows that compound 1 and 2 non-cytotoxic because IC_{50} value is very high.

CONCLUSION

Compound (1) and (2) exhibited antioxidant activity with IC_{50} 6.42 and 11.80 µg/mL by DPPH and by reducing power assay method with IC_{50} 7.02 and 13.74 µg/mL recpectively. Both compounds are non-cytotoxic because IC_{50} value is very high (above the NCI reference).

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The author declare there is no conflict interest in this research.

ORIGINALITY DECLARATION

This article has not been submitted or published elsewhere for publication

ABBREVIATION USED

DMEM: Dulbecco's Modified Eagle's Medium; **DPPH:** *1,1-Diphenyl-2-picrylhydrazyl radical, 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl*; **COSY:** correlation spectroscopy; **NOESY:** Nuclear Overhauser SpectroscopY; **HMBC**: Heteronuclear Multiple Bond Correlation).

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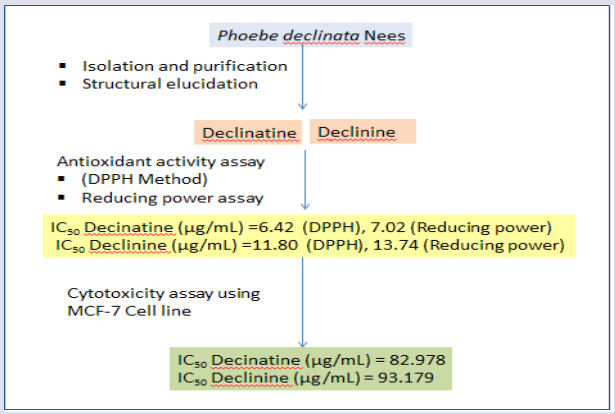
GRAPHICAL ABSTRACT



SUMMARY

- *Phoebe declinata* Nees belongs to Lauraceae family which commonly called in Indonesia as huruhejo or bedagai have been reported to produce isoquinoline alkaloids as aporphines, noraporphines, and benzylisoquinolines.
- Many of these isolates exhibit diversified biological activities, including anti-diabetes, anti-inflammation, cytotoxic, antibacterial, antifungal activities and antioxidant properties
- This research was the first study reported new alkaloid, declinatine and declinine, which have been isolated from *Phoebe declinata* Nees and its cytotoxicity to MCF-7 cell line.

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