

# Alkaloid from *Phoebe declinata* Nees Leaves

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## ABSTRACT

**Introduction:** Genus *Phoebe* have been reported to produce isoquinoline alkaloids as aporphines, noraporphines, and benzyloisoquinolines. 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* againsts 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<sub>50</sub> value of 6.42 and 11.80 µg/mL respectively and using reducing power method for 1 and 2 showed IC50 value of 7.02 and 13.74 µg/mL respectively. Compound (1) and (2) exhibited cytotoxic activity against MCF-7 cells with an IC<sub>50</sub> 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.<sup>1</sup> 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.<sup>1,2</sup> Genus *Phoebe* have been reported to produce isoquinoline alkaloids as aporphines, noraporphines, and benzyloisoquinolines.<sup>3-5</sup> Many of these isolates exhibit diversified biological activities, including anti-diabetes, anti-inflammation, cytotoxic, antibacterial, antifungal activities and antioxidant properties.<sup>3-6,7</sup> Previous paper, we reported the isolation of alkaloid declinine from stem bark of *Phoebe declinata*.<sup>8</sup> 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 <sup>1</sup>H-NMR and <sup>13</sup>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<sub>254</sub>), 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<sub>4</sub>OH, and exhaustively extracted with dichloromethane by reflux again. The residue was continue extracted with methanol. The hexane, CH<sub>2</sub>CL<sub>2</sub> 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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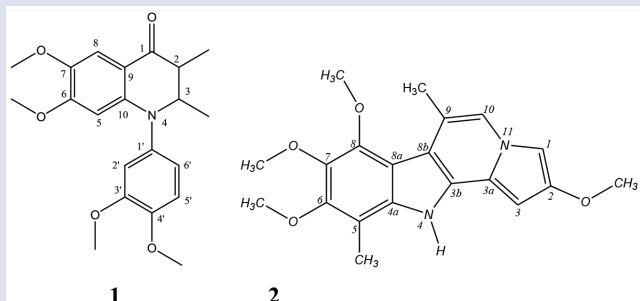


Figure 1: Isolated compounds from leaves of *Phoebe declinata*.

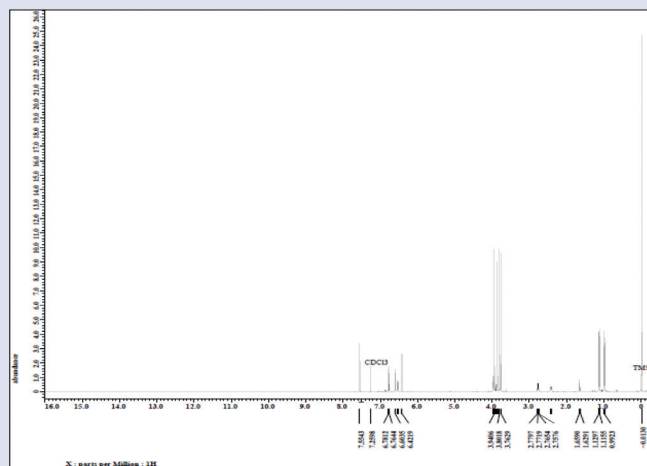


Figure S1: <sup>1</sup>H-NMR spectrum compound 1 in CDCl<sub>3</sub>.

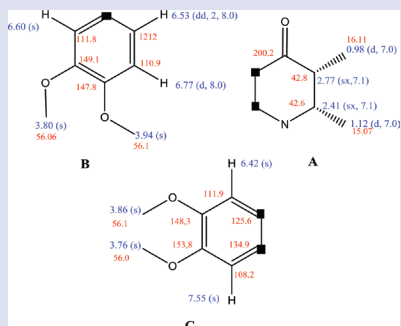


Figure 2: Partial Structures of A, B and C and <sup>1</sup>H, <sup>13</sup>C-Chemical shift data of Compound 1.

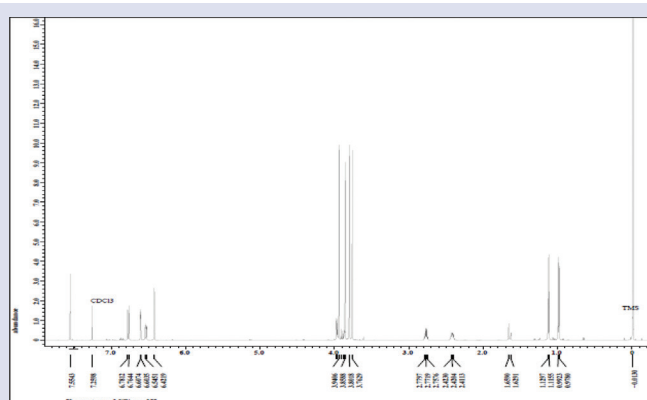


Figure S2: <sup>1</sup>H-NMR spectrum compound 1 in CDCl<sub>3</sub> (Expanded).

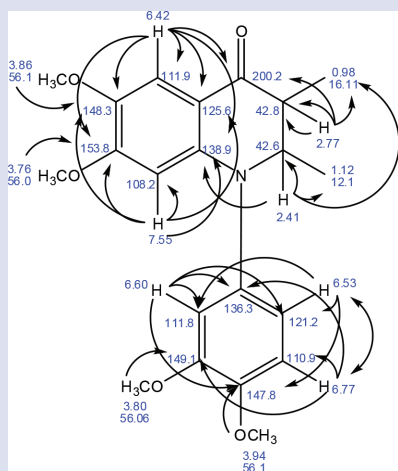


Figure 3: Selected HMBC correlation of Compound 1.

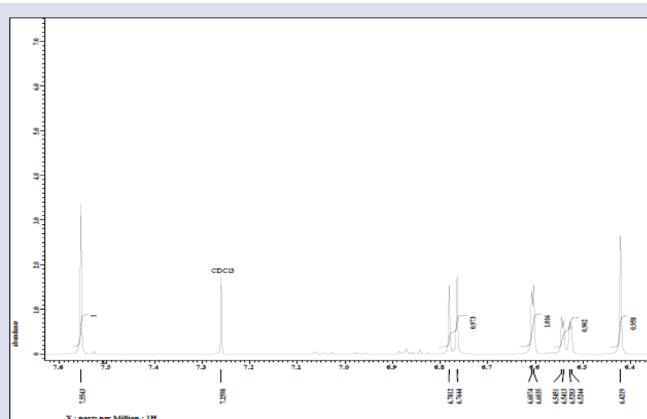


Figure S3: <sup>1</sup>H-NMR spectrum compound 1 in CDCl<sub>3</sub> (Expanded).

(DPPH).<sup>9</sup> 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:

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



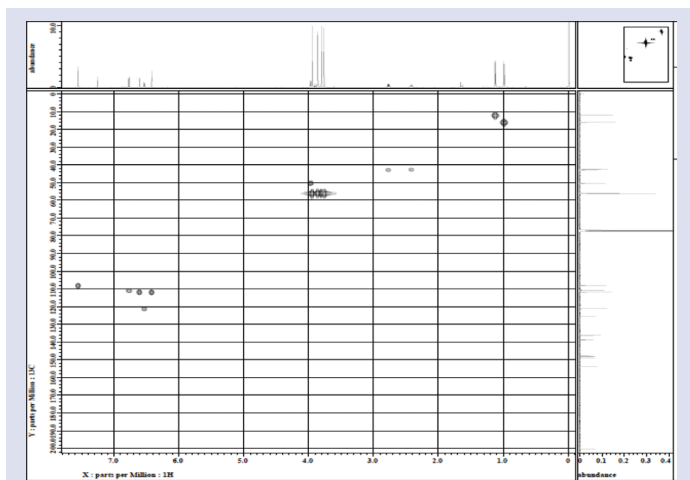


Figure S10: HMQC spectrum compound 1 in  $CDCl_3$ .

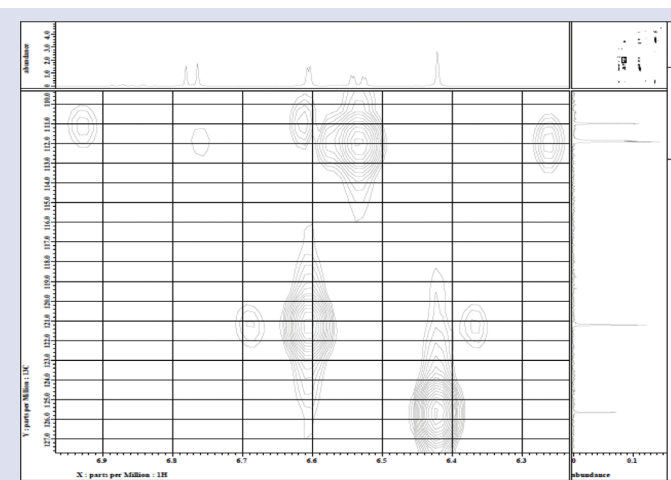


Figure S13: HMBC spectrum compound 1 in  $CDCl_3$  (Expanded).

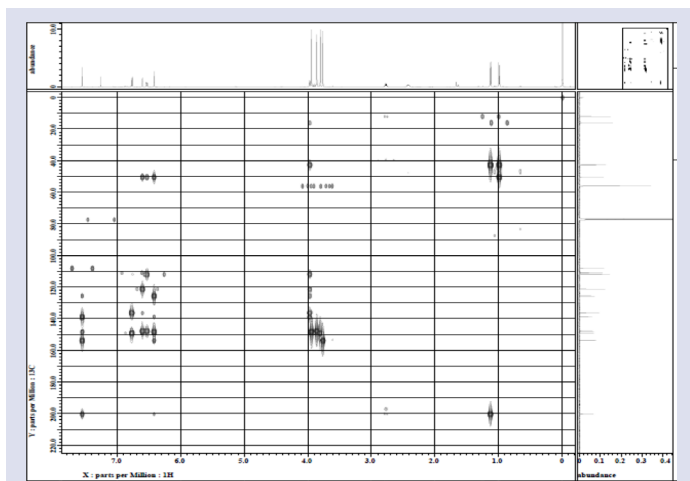


Figure S11: HMBC spectrum compound 1 in  $CDCl_3$ .

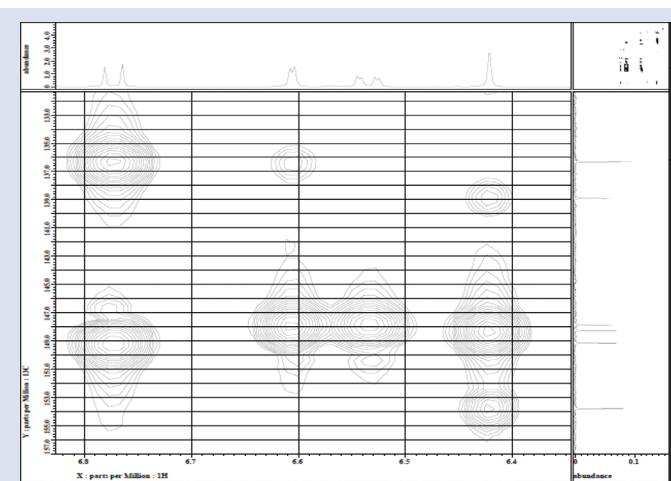


Figure S14: HMBC spectrum compound 1 in  $CDCl_3$  (Expanded).

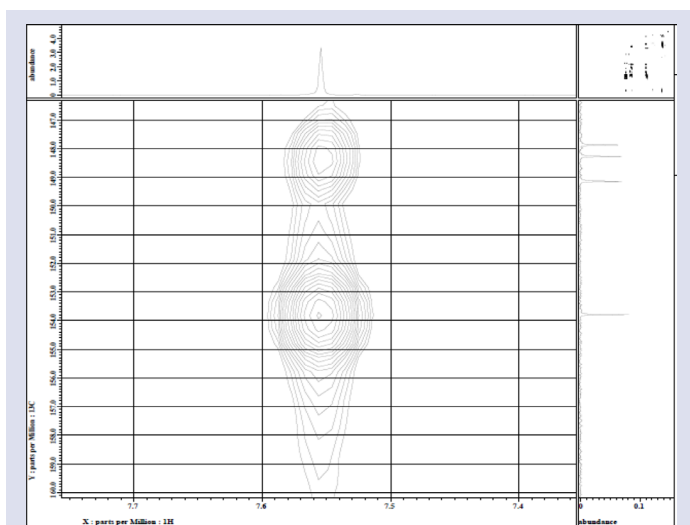


Figure S12: HMBC spectrum compound 1 in  $CDCl_3$  (Expanded).

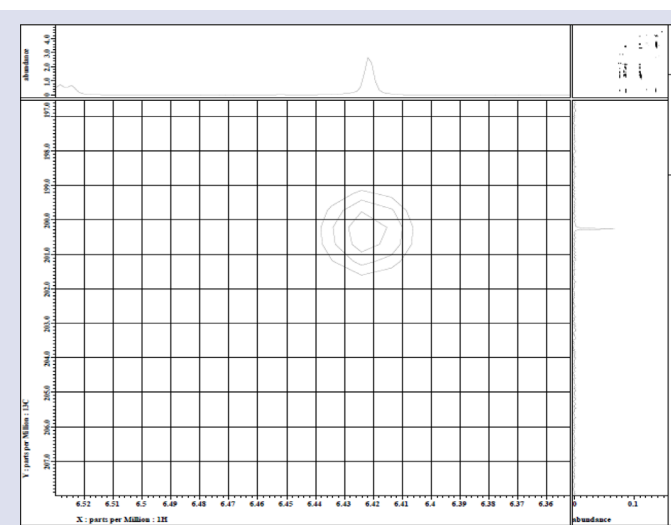


Figure S15: HMBC spectrum compound 1 in  $CDCl_3$  (Expanded).

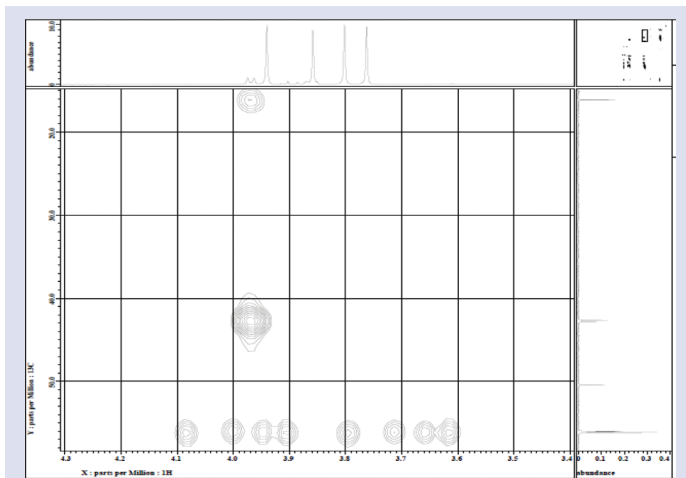


Figure S16: HMBC spectrum compound 1 in CDCl<sub>3</sub> (Expanded).

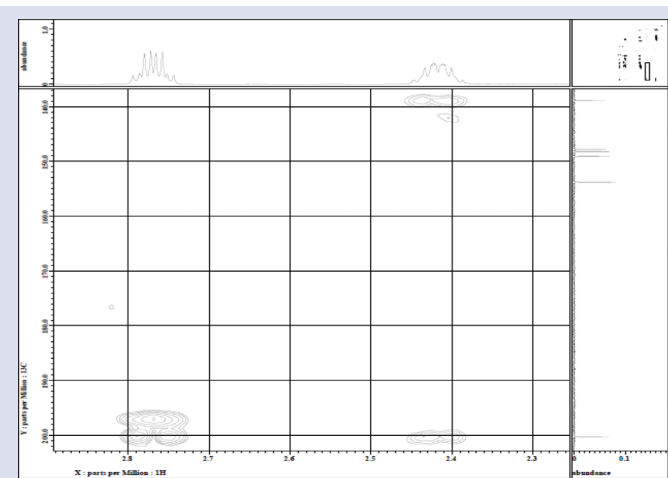


Figure S19: HMBC spectrum compound 1 in CDCl<sub>3</sub> (Expanded).

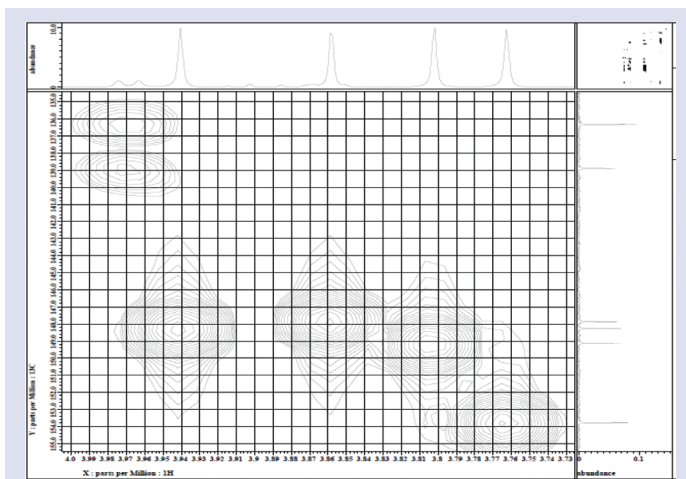


Figure S17: HMBC spectrum compound 1 in CDCl<sub>3</sub> (Expanded).

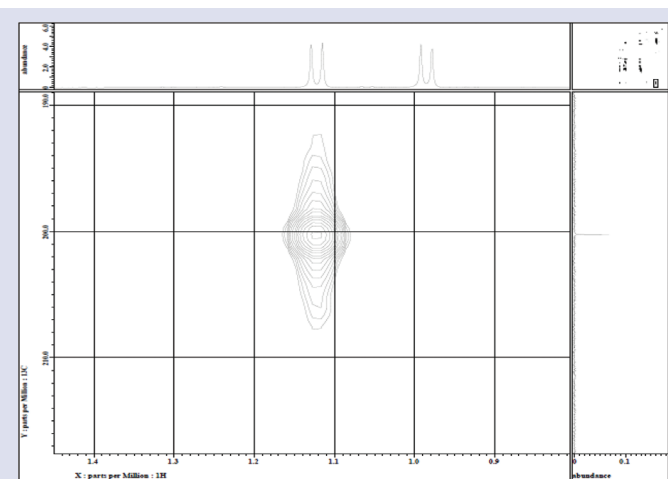


Figure S20: HMBC spectrum compound 1 in CDCl<sub>3</sub> (Expanded).

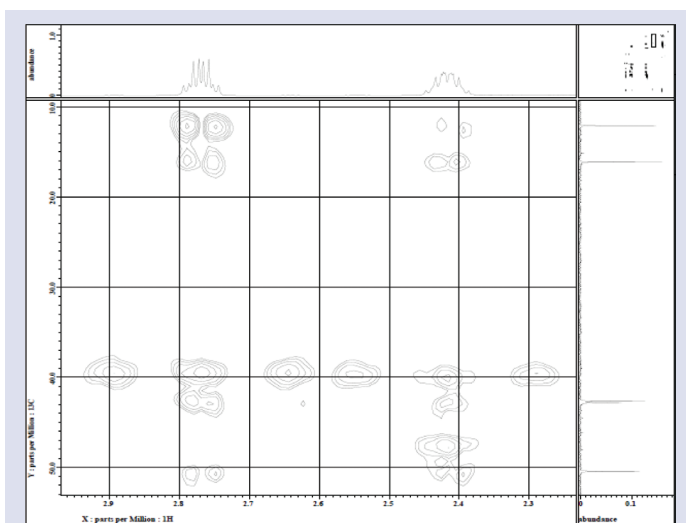


Figure S18: HMBC spectrum compound 1 in CDCl<sub>3</sub> (Expanded).

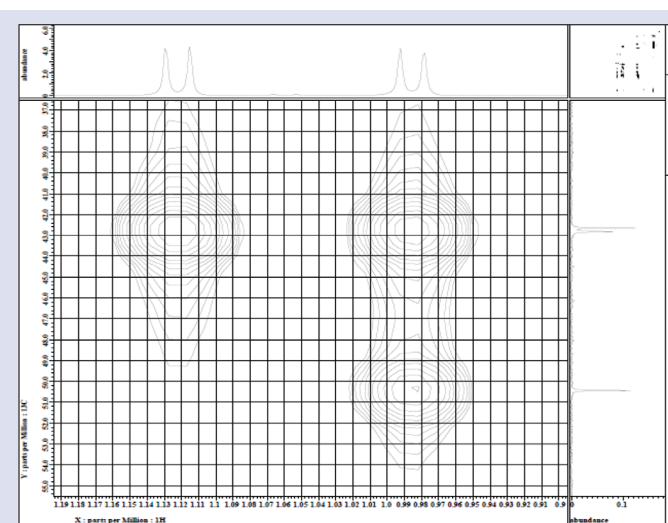


Figure S21: HMBC spectrum compound 1 in CDCl<sub>3</sub> (Expanded).





**Table 1:** <sup>1</sup>H-NMR and <sup>13</sup>C-NMR assignment for compound 1 in CDCl<sub>3</sub>.

No	δ <sub>H</sub>	δ <sub>C</sub>
1	-	200.2
2	2.77 (sektet, 7.1)	42.8
2-CH <sub>3</sub>	0.98 (d, 7.0)	16.11
3	2.41 (sektet, 7.1)	42.6
3-CH <sub>3</sub>	1.12 (d, 7.0)	12.10
5	7.55 (s)	108.2
6	-	153.8
6-OCH <sub>3</sub>	3.76 (s)	56.0
7	-	148.3
7-OCH <sub>3</sub>	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 <sub>3</sub>	3.80 (s)	56.06
4'	-	147.8
4'-OCH <sub>3</sub>	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

culated 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-3.

Compound 2 was obtained white crystal, m.p. 102-104°C, molecular formula C<sub>20</sub>H<sub>22</sub>N<sub>2</sub>O<sub>4</sub>. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, δ): 6.88 (*s*, CH-1), 7.07 (*s*, CH-3), 6.99 (*s*, CH-10), 3.86 (*s*, OCH<sub>3</sub>-2), 1.08 (*m*, CH<sub>3</sub>-5), 3.88 (*s*, OCH<sub>3</sub>-6), 3.89 (*s*, OCH<sub>3</sub>-7), 3.9 (*s*, OCH<sub>3</sub>-8), 0.67 (*m*, CH<sub>3</sub>-9) (Figure S23). <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 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<sub>3</sub>-2),

11.89 (CH<sub>3</sub>-5), 55.87 (OCH<sub>3</sub>-6), 55.95 (OCH<sub>3</sub>-7), 55.90 (OCH<sub>3</sub>-8), 15.05 (CH<sub>3</sub>-9) (Figure S24).

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

## CONCLUSION

Compound (1) and (2) exhibited antioxidant activity with IC<sub>50</sub> 6.42 and 11.80 µg/mL by DPPH and by reducing power assay method with IC<sub>50</sub> 7.02 and 13.74 µg/mL respectively. Both compounds are non-cytotoxic because IC<sub>50</sub> 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

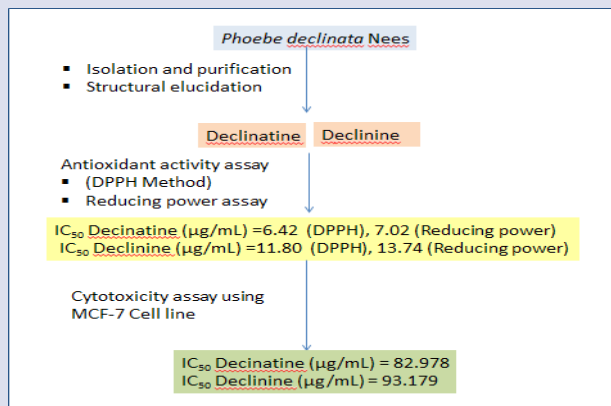
## ABBREVIATION USED

**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).

## REFERENCES

- Kostermans AJ, Lauraceae. Reinwardtia. 1957;4(2):193-256.
- Priyadi H, Takao G, Rahmawati I, Supriyanto B, Nursal WI. Five Hundred Plant Species in Gunung Halimun Salak National Park, West Java: A Checklist Including Sundanese Names, Distribution, and Use. CIFOR; 2010.
- Mukhtar MR, Aziz AN, Thomas NF, Hadi AH, Litaudon M, Grandine A, a new proaporphine alkaloid from the bark of *Phoebe grandis*. *Molecules*. 2009;14(3):1227-33.
- Semwal DK, Rawat U, Bamola A, Semwal R. Antimicrobial activity of *Phoebe lanceolata* and *Stephania glabra*; preliminary screening studies. *Journal of scientific research*. 2009;1(3):662-6.
- Awang K, Mukhtar MR, Hadi AH, Litaudon M, Latip J. New alkaloids from *Phoebe grandis* (Nees) Merr. *Natural product research*. 2006;20(6):567-72.
- Ayinde BA, Omogbai EK, Ikpefan EO. Comparative Cytotoxic and Antiproliferative Effect of *Persea Americana* Mill. (Lauraceae) Leaf, Stem and Root Barks. *Nigerian Journal of Pharmaceutical Science*. 2011;10(1):16-26.
- Yeh-Siang L, Subramaniam G, Hadi AH, Murugan D, Mustafa MR. Reactive oxygen species-induced impairment of endothelium-dependent relaxations in rat aortic rings: protection by methanolic extracts of *Phoebe grandis*. *Molecules*. 2011;16(4):2990-3000.
- Elya B, Forestrania RC, Harmita HO, Katrin R, Ulfah Z. Declinine: The new Alkaloid from *Phoebe declinata* Nees. *Int. Res. J Pharm*. 2014;5(4):271-4.
- Chang HY, Ho YL, Sheu MJ, Lin YH, GJ-Tseng MC. Antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts *Botanical Studies*. 2007;48:407-17.
- Doyle A, Griffiths JB. *Cell and Tissue Culture: Laboratory Procedures in Biotechnology*. Chichester: Wiley 2006.
- Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods*. 1983;65(1,2):55-63.

## 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 benzyloquinolines.
- 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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