INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

# DECLININE: THE NEW ALKALOID FROM *PHOEBE DECLINATA* NEES

Berna Elya[1]*, Roshamur C. Forestrania[1], Harmita[1], Hanita Omar[3,4], Katrin[1], Rosmalena[2] and Zakiyah Ulfah[1]
[1]Faculty of Pharmacy, University of Indonesia, Depok, Indonesia
[2]Faculty of Medicine, University of Indonesia, Depok 16424, Indonesia
[3]Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
[4]Centre for Foundation Studies in Science, University of Malaya, Kuala Lumpur 50603, Malaysia
*Corresponding Author Email: berna.elya@farmasi.ui.ac.id

Article Received on: 28/02/14 Revised on: 27/03/14 Approved for publication: 07/04/14

**DOI: 10.7897/2230-8407.050458**

**ABSTRACT**
The new alkaloid named as declinine had been isolated from stem bark of *Phoebe declinata* Nees (Lauraceae). This alkaloid had been obtained for the first time from this plant. The structure of declinine was determined by spectroscopic analysis of 1D and 2D NMR, IR, UV, and LCMS. Declinine exhibited good antioxidant activity by DPPH assay with an $IC_{50}$ value 11.8 µg/mL compared to boldine as a standard with an $IC_{50}$ value 5.8 µg/mL.
**Keywords**: *Phoebe declinata* Nees, antioxidant, alkaloid, declinine, DPPH

## INTRODUCTION

Indonesia hangs in the second position of the richest country in natural resources[1,2]. In addition, a total of therapeutic plants in Indonesia consist of almost 90 % of the therapeutics plants in the world[3]. But, the utilization of natural resources is still in minimum number that is roughly 1,000-1,200 species for traditional use and 300 species for industrial scale[1]. Thus, the development and exploration of natural resources in Indonesia is considerably needed. Lauraceae family, one of the sources of therapeutic plants, is widely distributed in Indonesia. The previous study reported that many of Lauraceae species exhibited various biological activities, such as antimicrobial, anticancer, antifungal, antidiabetic, and antioxidant activity[4-8]. One of the chemical constituents in Lauraceae which plays role as bioactive compound is alkaloid. Recently, researchers tend to investigate alkaloids as bioactive compound isolated from Phoebe, one of genus in Lauraceae. Studies revealed that the alkaloid from *Phoebe grandis* performed some of the biological activities, particularly as a potent antioxidant[9,10]. However, the study of *Phoebe* sp. is still remained for further investigation. *Phoebe declinata* Nees (Genus: Phoebe, Family: Lauraceae), popularly known as kayu helah or bedagai, is native plant species in Indonesia that spread out in Java and Sumatera. The plants are commonly evergreen as tall trees with small white flowers and fruits. The stems of these plants are white and they have the hard texture. Stem, barks, and leaves of this species possess particular smell. The chemical constituent preliminary study of *P. declinata* stem bark indicated that this species contained alkaloids. Thus, this research aimed to isolate and to elucidate the structure of alkaloid from stem bark of *P. declinata*. Based on the other studies of *Phoebe* species that had confirmed the potency of Phoebe alkaloid as an antioxidant agent, this research was intended to investigate antioxidant activity of alkaloid using DPPH method.

## MATERIAL AND METHODS

### General

The structure was determined by analysing the spectra of $^{1}$H-NMR (FT-NMR, JNM Lambda 400 Mhz, Jeol) and $^{13}$C-NMR (Ultrashield 100 Mhz Plus, Bruker) using Chloroform-d, LC-MS (6530, Accurate-Mass LC/MS, Agilent Technologies), and Infrared (FT-IR, Spectrum RX I, PerkinElmer). The column chromatography used silica gel 60 (70-230 mesh, E. Merck 1.07734) as stationary phase. Alkaloid spots were visualized by Dragendorff's spray method in aluminium sheet (20 × 20 cm Silica gel 60 F254) and examined under UV light with λ 254 and 366 nm (T80+, PG Instruments Ltd). Mayer's reagent, Dragendorff 's reagent and Bouchardat's reagents were used for alkaloid screening.

### Plant material

Stem bark of *P. declinata* (Lauraceae) was obtained from Bogor Botanical Garden West Java Indonesia and identified by Centre for Plant Conservation in Indonesian Scientific Knowledge, Bogor, Indonesia. Voucher specimen (PB 1065) is deposited at the Herbarium Faculty of Pharmacy University of Indonesia.

### Extraction and isolation of Declinine

Isolation process was attempted to obtain alkaloid which showed antioxidant activity. The dried stem bark of *P. declinata* (1.8 kg) was grinded and then saturated by 54 % $NH_4OH$ for 2 h. The plant material was dried and extracted six times using hexane in 30 minutes for each extraction cycle. The plant's residue from the first extraction was saturated again by 54 % $NH_4OH$. Then, it was dried and afforded into the next exhaustive extraction using $CH_2Cl_2$ until it didn't indicate the presence of alkaloid. The $CH_2Cl_2$ extract was evaporated using rotary evaporator to eliminate the remained solvent (24.0 g) and subjected into the first column chromatography using silica gel as stationary phase and $CH_2Cl_2$-methanol as mobile phase. The first column chromatography was carried out with certain ratios of both

solvents by gradient system. It yielded 12 fractions as a result of combining the similarities profile in their separation through TLC method. The sixth fractions (0.8 g) which had the highest antioxidant activity was attempted to the next fractionation step which is the second column chromatography by gradient system using hexane-ethyl acetate and silica gel. The fourth fraction (137 mg) of the total 8 fractions from the second column chromatography was purified by crystallization. The pure compound was named declinine (61, 6 mg). The antioxidant activity of declinine was examined by measuring its activity in scavenging stable free radical DPPH. Its activity was represented as $IC_{50}$. Declinine (61.6 mg): white crystal, m.p. 102–104°C, molecular formula $C_{20}H_{22}N_2O_4$. $^1$H-NMR ($CDCl_3$ $\delta$): 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 ($OCH_3$-6), 3.89 ($OCH_3$-7), 3.9 ($OCH_3$-8), 0,67 (*m*, $CH_3$-9).$^{13}$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), 55.86 ($OCH_3$-6), 55.95 ($OCH_3$-7), 55.90 ($OCH_3$-8), 15.05 ($CH_3$-9).

**Antioxidant activity**

The antioxidant activity of extracts, fractions, and isolate were assessed by measuring their scavenging potency against stable free radical 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH)[11]. 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, 8, 9, and 10 µg/mL) were added into mixed solution at the separated place. The reaction mixtures were incubated in the dark at temperature 37ºC for 30 minutes. 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:

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

Where A is the absorbance of blank or sample.

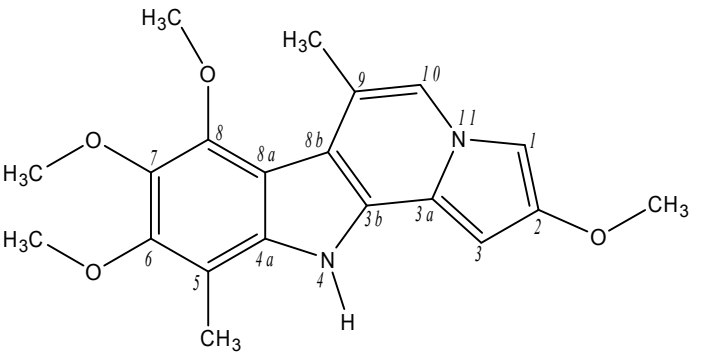


**Figure 1: Structure of declinine**

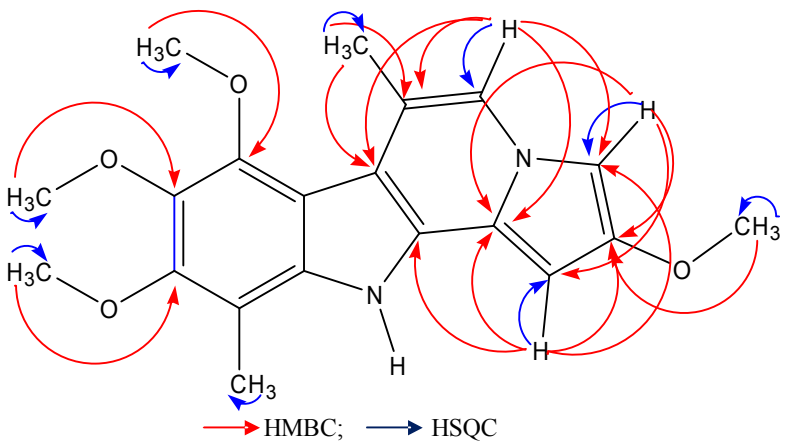


**Figure 2: Key HSQC and HMBC correlations (H → C) of declinine**

**Table 1: $^{1}$H- and $^{13}$C-NMR spectrum of declinine**

| Position | Chemical shift (δ) (ppm) | | DEPT | HMBC |
|---|---|---|---|---|
| | $^{1}$H-NMR | $^{13}$C-NMR | | |
| 1 | 6.88 (1H s) | 118.51 | CH | 2, 3, 3a |
| 2 | - | 148.99 | C | - |
| 3 | 7.07 (1H s) | 110.38 | CH | 3b, 3a, 2, 1 |
| 3a | - | 147.90 | C | - |
| 3b | - | 135.69 | C | - |
| 4a | - | 147.78 | C | - |
| 5 | - | 133.48 | C | - |
| 6 | - | 148.60 | C | - |
| 7 | - | 148.96 | C | - |
| 8 | - | 148.64 | C | - |
| 8a | - | 134.84 | C | - |
| 8b | - | 133.26 | C | - |
| 9 | - | 133.82 | C | - |
| 10 | 6.99 (1H s) | 109.35 | CH | 9, 8b, 1, 3a |
| 2-$OCH_3$ | 3.86 (3H s) | 55.88 | $CH_3$ | 2 |
| 5-$CH_3$ | 1.08 (3H m) | 11.89 | $CH_3$ | - |
| 6-$OCH_3$ | 3.88 (3H s) | 55.86 | $CH_3$ | 6 |
| 7- $OCH_3$ | 3.89 (3H s) | 55.95 | $CH_3$ | 7 |
| 8- $OCH_3$ | 3.9 (3H s) | 55.90 | $CH_3$ | 8 |
| 9-$CH_3$ | 0,67 (3H m) | 15.05 | $CH_3$ | 9, 8b |

$^{1}$H- (400 MHz) and $^{13}$C- NMR (100 MHz), δ ppm, in $CDCl_3$

**Table 2: DPPH scavenging activity of alkaloid fractions**

| Alkaloid Fractions | $IC_{50}$ µg/mL |
|---|---|
| A-5 | 256.8 |
| A-6 | 68.9 |
| A-8 | 92.2 |
| A-9 | 180.8 |
| Boldine | 5.8 |

**Table 3: DPPH scavenging activity of declinine**

| Sample | $IC_{50}$ µg/mL |
|---|---|
| Declinine | 11.8 |
| Boldine | 5.8 |

## RESULTS AND DISCUSSION

### Structural analysis

Decilinine or 2, 6, 7, 8-tetramethoxy-5, 9-dimethyl-11H-indolizidino [8, 7-b]indole (Figure 1) was obtained as white crystal with melting point range 102-104$^{o}$C. UV-Vis spectra of declinine emerged $\lambda_{max}$ at 210, 232, and 279 nm which indicated the existence of the indole chromophore of indole alkaloid[12]. IR spectrum revealed peaks at 295.66 and 2835.28 $cm^{-1}$ that suggested the presence of methoxy groups. The –NHR group was noticed as a peak at 3100 $cm^{-1}$. The aromatic structure appeared as stretching of =C–H (aromatic) at 3000 $cm^{-1}$ and stretching of C=C (cyclic) at 1592.13 and 1516.26 $cm^{-1}$. The Mass spectrum of declinine showed peak at m/z 355.19 $[M+H]^{+}$ which corresponded to a molecular formula $C_{20}H_{22}N_2O_4$ (calcd for $C_{20}H_{23}N_2O_4$, 355.19). The $^{1}$H-NMR spectrum revealed a singlet at δ 1.62 (1H, s) that suggested the presence of –NH and four singlet at δ 3.86 (3H, s), δ 3.88 (3H, s), δ 3.89 (3H, s), and δ 3.91 (3H, s) that represented –$OCH_3$. While the aromatic signal were at δ 6.88 (1H, m), δ 6.99(1H, m), and δ 7.07 (1H, m), and hydrogen of –$CH_3$ attaching in aromatic areas emerged as singlet peaks at δ 1.80 (3H, s) and δ 1.78 (3H, s). The $^{13}$C-NMR spectrum indicated the presence of 20 carbons. The $^{13}$C-NMR analysis gave the information about the appearance of four methoxy groups at δ 55.86; δ 55.88; δ 55.90; and δ 55.95, two methyl groups at δ 11.89 and δ 15.05, three aromatic groups at δ 109.34; δ 110.38; δ 118.51, and eleven quaternary carbons at δ 133-149. HMBC analysis (Figure 2) suggested the certain positions of methoxy groups by the correlations of H-(2-$OCH_3$) with C-2, H-(6-$OCH_3$) with C-6, H-(7-$OCH_3$) with C-7, and H-(8-$OCH_3$) with C-8. Cross peaks between H-3 with C-3b, C-3a, C-2, C-1; H-1 with C-2, C-3, C-3a; and H-10 with C-9, C-8b, C-1, C-3a also confirmed the structure of declinine. Furthermore, the overall structure of declinine was supported by HSQC and HMBC data (Table 1, Figure 2).

### Antioxidant activity

$CH_2Cl_2$ extract from stem bark of *P. declinata* performed higher antioxidant potency ($IC_{50}$ 13.5 µg/mL) than antioxidant potency of hexane extract ($IC_{50}$ 28.3 µg/mL). Because hexane extract had lower potency than the potency of dichloromethane extract, the next isolation step was afforded to $CH_2Cl_2$ extract. The first column chromatography of $CH_2Cl_2$ extract produced 4 fractions, and antioxidant activity in A-6 fraction was measured to be the most potent antioxidant among them (Table 2). Fraction A-6 was afforded to the further fractionation step in second column chromatography. Alkaloid, named as declinine, was found as crystal which came out from the first fraction of second column chromatography. Declinine was considered as a good antioxidant agent with $IC_{50}$ 11.8 µg/mL which is compared to boldine as alkaloid standard with $IC_{50}$ 5.8 µg/mL (Table 3) by the DPPH method.

**ACKNOWLEDGEMENTS**

The authors are thankful to the BOPTN 2013 University of Indonesia for funding this research and University of Malaya Malaysia for providing the instruments to conduct this research.

**REFERENCES**

1. Department of Health RI. Kotranas. Jakarta Indonesia: Department of Health Republic Indonesia; 2007. p. 14.
2. Tarigan IM, Bahri S, Saragih A. Antihyperuricemic Activity of Ethanol Extract of Suruhan Herb (*Peperomia pellucida* (L.) Kunth) in Male Mice. Journal of Pharmaceuticals and Pharmacology 2012; 1: 37–43.

3. Nugroho IA. Lokakarya Nasional Tanaman Obat Indonesia. 2nded. Bogor, Indonesia: Badan Penelitian dan Pengembangan Kehutanan; 2010. p. 1.
4. Chaverri C, Díaz C, Cicció F. Chemical Analysis of Essential Oils from *Ocotea gomezii* W.C. Burger and Ocotea morae Gómez-Laur. (Lauraceae) Collected at Reserva Biológica Alberto M. Brenes in Costa Rica and their Cytotoxic Activity on Tumor Cell Lines. Brazil Chemistry Society 2011; 22: 741–745. http://dx.doi.org/10.1590/S0103-50532011000400018
5. Ayinde BA, Omogbai EKI, Ikpefan EO. Comparative Cytotoxic and Antiproliferative Effect of *Persea americana* Mill. (Lauraceae) Leaf, Stem and Root Barks. Nigerian Journal of Pharmaceutical Science 2011; 10: 16–26.
6. Kumanan R, Manimaran S, Saleemulla K, Dhanabal SP, Nanjan MJ. Screening of Bark *Cinnamomum tamala* (Lauraceae) by Using a-amylase Inhibition Assay for Anti-diabetic Activity. Pharmaceutical and Biomedical Research 2010; 1: 69–72.
7. Gomez Flores R, Arzate Quintana C, Quintanilla Licea R, Tamez Guerra P, Tames Guerra R, Monreal Cuevas E, Rodriguez Padilla. Antimicrobial Activity of *Persea americana* Mill (Lauraceae) (Avocado) and *Gymnosperma glutinosum* (Spreng.) Less (Asteraceae) Leaf Extracts and Active Fractions against *Mycobacterium tuberculosis*. American-Eurasian Journal of Science Research 2008; 3: 188–194.
8. Shahwar D, Shafiq Ur Rehman, Ahmad N, Ullah S, Raza MA. Antioxidant Activities of the Selected Plants from the Family Euphorbiaceae, Lauraceae, Malvaceae, and Balsaminaceae. African Journal of Biotechnology 2010; 9: 1086–1096.
9. O'Brien P, Carrasco Pozo C, Speisky H. Boldine and Its Antioxidant or Health Promoting Properties. Chemical-Biological Interactions 2006; 159: 1–17. http://dx.doi.org/10.1016/j.cbi.2005.09.002
10. Mukhtar MR, Aziz AN, Thomas NF, Hadi AH, Litaudon M, Awang K. A New Proaporphine Alkaloid from The Bark of *Phoebe grandis*. Molecules 2009; 14: 1227–1233. http://dx.doi.org/10.3390/molecules14031227
11. Molyneux P. The Use of The Stable Free Radical Diphenylpicrylhydrazyl (DPPH) for Estimating Antioxidant Activity. Songklanakarin Journal of Science Technologies 2004; 26: 211–219.
12. Tan SJ, Choo Y, Thomas NF, Robinson WT, Komiyama K, Kam TS. Unusual Indole Alkaloid-pyrrole, -pyrone and -carbamic acid adducts from *Alstonia angustifolia*. Tetrahedon 2010; 66: 7799–79806. http://dx.doi.org/10.1016/j.tet.2010.07.079

**Cite this article as:**
Berna Elya, Roshamur C. Forestrania, Harmita, Hanita Omar, Katrin, Rosmalena and Zakiyah Ulfah. Declinine: The new alkaloid from Phoebe declinata Nees. Int. Res. J. Pharm. 2014; 5(4):271-274 http://dx.doi.org/10.7897/2230-8407.050458

Source of support: BOPTN 2013 University of Indonesia, Conflict of interest: None Declared