The Journal of Chulabhorn Royal Academy
eISSN : 2697-5203 (Online)

Research article

# Chemical Constituents and their Antifungal Activity from the Branch Extracts of *Plumbago indica* L.

**Sirawit Manyaem[1], Maneekarn Namsa-ard[1]*, Srisombat Puttikamonkul[2], Surasak Prachya[3], Anucha Namsa-ard[4], Siritron Samosorn[1]**

[1]*Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand*
[2]*Department of Microbiology, Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand*
[3] *Laboratory of Natural Products, Chulabhorn Research Institute, Bangkok, Thailand*
[4]*Chulabhorn Royal Academy, Bangkok, Thailand*

*Corresponding Author, e-mail: maneekarn@g.swu.ac.th

Received: 26 June 2022; Revised: 8 August 2022; Accepted: 6 September 2022

## Abstract

**Background:** *Plumbago indica* L. produces bioactive compounds with anti-inflammatory, antifungal, antibacterial, antiparasitic, antifertility, anti-oxidant, wound healing, anticancer and antiviral activities. The study aimed to isolate, separate and evaluate compounds' antifungal activities from the branches of *P. indica.*

**Methods:** The crude dichloromethane and ethyl acetate extracts of branches of *P. indica* were individually separated and purified by various column chromatographic techniques. The structures were elucidated using spectroscopic techniques. The isolated compounds were evaluated for antifungal activity against *Aspergillus flavus* and *Talaromyces marneffei.*

**Results:** Plumbagin **(1)**, *β*-sitosterol **(2)**, maritinone **(3)** and 7–7′-biplumbagin **(4)** were isolated from dichloromethane extract, while *cis*-isoshinanolone **(5)** and *trans*-isoshinanolone **(6)** were isolated from the ethyl acetate extract of the branches of *P. indica.*

Compound **1** antifungal activity against *A. flavus* and *T. marneffei* with zones of inhibition of 40.0 and 70.0 mm (at 1.0 mg/disc). Compound **5** exhibited antifungal activity against *A. flavus* and *T. marneffei* with zones of inhibition of 7.0 and 12.0 mm, respectively (at concentrations of 1.0 and 0.25 mg/disc), and compound **6** showed activity against *A. flavus* and *T. marneffei* with zones of inhibition of 7.0 and 10.0 mm, respectively (at concentrations of 1.0 and 0.25 mg/disc). Compounds **3, 4** and **6** were obtained from this plant for the first time.

**Conclusions:** Six constituents, plumbagin **(1)**, β-sitosterol **(2)**, maritinone **(3)**, 7–7′-biplumbagin **(4)**, *cis*-isoshinanolone **(5)** and *trans*-isoshinanolone **(6)**, were isolated from the branches of *P. indica*. From these results, plumbagin can be considered the active chemical constituent responsible for the antifungal activity against *A. flavus* and *T. marneffei.* This study is the first to report the isolation of compounds **3, 4** and **6** from *P. indica* and the antifungal activity of compounds **1**, **5** and **6** against *A. flavus* and *T. marneffei.*

**Keywords:** *Plumbago indica*, *Plumbaginaceae*, Plumbagin, Antifungal Activity, Medicinal Plant


© Chulabhorn Royal Academy. Terms and conditions of Creative Commons Attribution 4.0 International (CC BY NC ND 4.0) apply

## Introduction

The genus *Plumbago* belongs to the Plumbaginaceae family which consists of approximately 24 genera and 400 species[1]. *Plumbago indica* L. is widely distributed in the tropical and subtropical regions of Africa, Australia, and Asia including Thailand. *P. indica*, known as Chettamuun Phloeng Daeng, is commonly used in Thai traditional medicine where its root extract is used to prepare adaptogenic drugs for cancer patients[2]. Studies on the pharmacological properties of *P. indica* have shown a wide range of biological activities, such as anti-inflammatory[3], anticancer[2,4-6], antimicrobial[7-9], antioxidant[10], antidiarrheal[11-12], antifertility[13], antifungal[14] and wound healing activities[15-16]. Previous phytochemical investigations on *P. indica* have revealed an array of structurally diverse quinones, alkaloids, flavonoids, saponins, glycosides and tannins[17]. Preliminary bioactivity tests of the crude extract revealed that a dichloromethane extract from *P. indica* showed antifungal activity against *Aspergillus flavus* and *Talaromyces marneffei*. *A. flavus* produces aflatoxin and poses a significant risk of liver cancer while *T. marneffei* is considered to commonly cause infection in individuals with human immunodeficiency virus (HIV) infection. The present study will describe the isolation and biological evaluation of metabolites from the branches of *P. indica*. This is the first report of compounds **3, 4** and **6** from *P. indica*.

## Method

### General experimental procedures

**Nuclear Magnetic Resonance** (NMR) spectra were recorded on a Bruker AVANCE III HD system (Billerica, MA, USA) (400 MHz for $^{1}$H-NMR, 100 MHz for $^{13}$C-NMR) and Bruker AVANCE III (500 MHz for $^{1}$H-NMR, 125 MHz for $^{13}$C-NMR). The HR TOF mass spectra were obtained using a microtOF Bruker spectrometer. Column chromatography was performed using silica gel (< 63 µm, 40-63 µm and 63-200 µm, Merck, Whitehouse, NJ, USA), Sephadex LH-20 (Amersham Biosciences) and reversed-phase C18 silica gel (40-63 µm, Merck. Analytical thin-layer chromatography (TLC) was performed using precoated silica gel 60 GF254 plates (Merck).

### Plant material

Dried branches of *P. indica* L. were purchased from a herbal pharmacy (TPC-HERB, Bangkok, Thailand) in 2017. A voucher specimen has been deposited at the Department of Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok, Thailand.

### Fungal materials

*Aspergillus flavus* (TISTR 3041) was obtained from the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. *Talaromyces marneffei* (ATCC18224) was purchased from the Microbiology Department, Faculty of Medicine, Chiang Mai University, Thailand.

### Extraction and isolation

Extracts were obtained from the dried branches of *P. indica* using dichloromethane ($CH_2Cl_2$) and ethyl acetate (EtOAc) five times, each extraction for 2 d at room temperature. Evaporation of the filtrate under reduced pressure gave 5.47 g of $CH_2Cl_2$ extract and 78.31 g of EtOAc extract. The $CH_2Cl_2$ extract was subjected to silica gel column chromatography (CC) using a gradient of hexane-EtOAc (10:0 to 3:7) to obtain 10 fractions ($C_1$-$C_{10}$). Fraction $C_2$ (581.5 mg) was purified by silica gel CC eluting with a gradient of hexane–$CH_2Cl_2$ (9:1 to 0:10) to yield compound **1** (156.5 mg). Fraction $C_4$ (398.5 mg) was separated by silica gel CC eluting with hexane-EtOAc (9.8:0.2 to 0:10), yielding 19 fractions ($C_{4,1}$-$C_{4,19}$). Fraction $C_{4,5}$ (184.2 mg) was crystallized from $CH_2Cl_2$-MeOH to obtain compound **2** (70.0 mg). Fraction $C_{4,6}$ (81.6 mg) was further purified by silica gel CC eluting with hexane-EtOAc (9.6:0.4) to yield compound **3** (17.0 mg). Fraction $C_5$ (900.3 mg) was subjected to silica gel CC eluting with hexane–$CH_2Cl_2$ (6:4 to 0:10) to give 11 fractions ($C_{5,1}$-$C_{5,11}$). Fraction $C_{5,4}$, $C_{5,5}$ and $C_{5,6}$ (64.2 mg) were combined then fractionated by silica gel CC with hexane–$CH_2Cl_2$ (1:1 to 0:10) to provide compound **4** (1.5 mg). The EtOAc extract (78.31 g) was subjected to silica gel CC eluting with $CH_2Cl_2$-MeOH (10:0 to 1:1) to obtain 14 fractions ($E_1$-$E_{14}$). Fraction $E_8$ (2.2509 g) was separated by silica gel CC with hexane

-EtOAc (9.5:0.5 to 7:3), yielding 15 fractions ($E_{8,1}$-$E_{8,15}$). Fraction $E_{8,11}$ (242.6 mg) was further purified by silica gel CC eluting with hexane–$CH_2Cl_2$ (2:8) to give 15 fractions ($E_{8,11,1}$- $E_{8,11,15}$). Fraction $E_{8,11,4}$ (156.0 mg) was further purified by size exclusion CC on Sephadex LH-20 eluting with $CH_2Cl_2$-MeOH (3:7) to provide 4 fractions ($E_{8,11,4,1}$-$E_{8,11,4,4}$). Fraction $E_{8,11,4,3}$ (142.2 mg) was fractionated by silica gel CC with hexane-EtOAc (1:9) to yield compound **5** (15.7mg) and compound **6** (14.8 mg).

**Characteristics of compounds**

Compound **1**: red solid; ESITOF-MS: *m/z* 187.0405 $[M-H]^-$, calculated for $C_{11}H_7O_3$ 187.0401. $^1$H-NMR ($CDCl_3$) $\delta_H$: 11.97 (1H, *s*, 5-OH), 7.63 (1H, *dd*, *J* = 8.1, 1.4 Hz, H-8), 7.60 (1H, *dd*, *J* = 8.1, 7.5 Hz, H-7), 7.25 (1H, *dd*, *J* = 7.5, 1.4 Hz, H-6), 6.80 (1H, *q*, *J* = 1.5 Hz, H-3), 2.19 (3H, *d*, *J* = 1.5 Hz, 11-$CH_3$). $^{13}$C-NMR ($CDCl_3$) $\delta_C$ : 190.2 (C-4), 184.7 (C-1), 161.1 (C-5), 149.6 (C-2), 136.1 (C-7), 135.4 (C-3), 132.0 (C-9), 124.1 (C-6), 119.2 (C-8), 115.1 (C-10), 16.5 (11-$CH_3$).

Compound **2**: white solid; ESITOF-MS:*m/z* 437.3750 $[M+Na]^+$, calculated for $C_{29}H_{50}ONa$, 437.3754. $^1$H-NMR ($CDCl_3$) $\delta_H$: 5.35 (1H, *d*, *J* = 5.3 Hz, H-6), 3.52 (1H, *m*, H-3), 1.00 (3H, *s*, H-29), 0.93 (3H, *d*, *J* = 6.5 Hz, H-21), 0.86 (3H, *t*, *J* = 7.2 Hz, H-29), 0.81 (3H, *d*, *J* = 6.6 Hz, H-26 and H-27). $^{13}$C-NMR ($CDCl_3$) $\delta_C$ : 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.1 (C-9), 45.9 (C-22), 42.3 (C-13), 42.3 (C-4), 39.8 (C-12), 37.3 (C-1), 36.5 (C-29), 36.1 (C-10), 34.0 (C-20), 31.9 (C-7), 31.9 (C-8), 31.7 (C-2), 29.2 (C-25), 28.2 (C-16), 26.1 (C-21), 24.3 (C-15), 23.1 (C-23), 21.1 (C-11), 19.8 (C-28), 19.4 (C-27), 19.0 (C-19), 18.8 (C-26), 12.0 (C-29), 11.9 (C-24).

Compound **3**: red solid; ESITOF-MS: *m/z* 397.0677 $[M+Na]^+$, calculated for $C_{22}H_{14}O_6Na$, 397.0683. $^1$H-NMR ($CDCl_3$) $\delta_H$: 12.58 (1H, *s*, 5-OH), 7.20 (1H, *d*, *J* = 8.7 Hz, H-7), 7.29 (1H, *d*, *J* = 8.7 Hz, H-6), 6.81 (1H, *q*, *J* = 1.4 Hz, H-3), 2.01 (3H, *d*, *J* = 1.4 Hz, 11-$CH_3$). $^{13}$C-NMR ($CDCl_3$) $\delta_C$ : 190.5 (C-4), 185.2 (C-1), 161.3 (C-5), 150.0 (C-2), 138.0 (C-7), 134.9 (C-3), 128.2 (C-9), 124.3 (C-6), 135.6 (C-8), 115.5 (C-10), 16.6 (11-$CH_3$).

Compound **4**: red solid; ESITOF-MS: *m/z* 375.0861 $[M+H]^+$, calculated for $C_{22}H_{15}O_6$, 375.0855. $^1$H-NMR ($CDCl_3$) $\delta_H$: 12.5 (1H, *s*, 5-OH), 7.73 (1H, *s*, H-8), 7.73 (1H, *s*, H-8), 6.85 (1H, *q*, *J* = 1.3 Hz, H-3), 2.22 (3H, *d*, *J* = 1.3 Hz, 11-$CH_3$). $^{13}$C-NMR ($CDCl_3$) $\delta_C$: 190.5 (C-4), 184.5 (C-1), 158.9 (C-5), 149.8 (C-2), 131.2 (C-7), 135.5 (C-3), 131.9 (C-9), 118.7 (C-6), 137.7 (C-8), 115.3 (C-10), 16.5 (11-$CH_3$).

Compound **5**: colorless oil; ESITOF-MS: *m/z* 191.0711 $[M-H]^-$, calculated for $C_{11}H_{11}O_3$ 191.0714). $^1$H-NMR ($CDCl_3$) $\delta_H$: 12.40 (1H, *s*, 8-OH), 7.47 (1H, *dd*, *J* = 7.5, 8.3 Hz, H-6), 6.92 (1H, *dd*, *J* = 8.4, 1.0 Hz, H-5), 6.91 (1H, *dd*, *J* = 7.4 Hz, H-7), 4.73 (1H, *br d*, *J* = 2.4 Hz, H-4), 2.85 (1H, *dd*, *J* = 11.0, 17.6 Hz, H-2a), 2.55 (1H, *dd*, *J* = 17.6, 4.3 Hz, H-2b), 2.42 (1H, *m*, H3), 1.98 (1H, *d*, *J* = 3.0 Hz, 4-OH), 1.16 (3H, *d*, *J* = 6.9 Hz, 11-$CH_3$). $^{13}$C-NMR ($CDCl_3$) $\delta_C$: 204.9 (C-1), 162.8 (C-8), 145.2 (C-10), 137.1 (C-6), 118.8 (C-5), 118.2 (C-7), 115.1 (C-9), 71.2 (C-4), 40.8 (C-2), 34.5 (C-3), 16.3 (11-$CH_3$).

Compound **6**: white solid; ESITOF-MS: *m/z* 191.0715 $[M-H]^-$, calculated for $C_{11}H_{11}O_3$ 191.0714). $^1$H-NMR ($CDCl_3$) $\delta_H$: 12.36 (1H, *s*, 8-OH), 7.50 (1H, *t*, *J* = 8.0 Hz, H-6), 7.11 (1H, *d*, *J* = 7.5 Hz, H-5), 6.90 (1H, *d*, *J* = 8.3 Hz, H-7), 4.50 (1H, *br d*, *J* = 7.4 Hz, H-4), 2.91 (1H, *dd*, *J* = 4.1, 17.4 Hz, H-2a), 2.43 (1H, *dd*, *J* = 17.4, 10.15 Hz, H-2b), 2.27 (1H, *m*, H3), 2.18 (1H, *d*, *J* = 6.5 Hz, 4-OH), 1.18 (3H, *d*, *J* = 6.4 Hz, 11-$CH_3$). $^{13}$C-NMR ($CDCl_3$) $\delta_C$: 203.9 (C-1), 162.6 (C-8), 146.0 (C-10), 137.2 (C-6), 117.4 (C-5), 117.3 (C-7), 115.4 (C-9), 73.8 (C-4), 43.5 (C-2), 37.6 (C-3), 17.9 (11-$CH_3$).

**Antifungal assay**

The antifungal assay used a modified disc diffusion method as previously described[18]. *A. flavus* (TISTR 3041) was grown on Potato Dextrose Agar (HiMedia, Mumbai, India) at 28 °C for 7 d, while *T. marneffei* (ATCC) was cultivated on Malt Extract Agar (HiMedia) at 28 °C for 14 d. Briefly, the fungi were harvested in 0.01 % Tween 80 by scraping with a sterile cotton swab then filtering through cotton wool. The fungal conidia were counted and diluted to 1 x $10^5$ conidia/mL. One hundred µL of 1 x $10^4$ conidia were used for the pour agar plate assay. The extract was diluted to obtain 20 µL of 0.25, 0.5 and 1 mg then spotted on a paper disc placed on a plate containing the target fungus. The control disc was spotted with 20 µL of DMSO. The antifungal activity of each

extract on the fungus was measured after incubation for 2 and 3 d at 28 ˚C and defined as the diameter of the inhibition zone.

## Results and Discussion

Purification of a crude dichloromethane extract of the branches of *P. indica* using silica gel column chromatography gave four compounds, including plumbagin **(1)**, β-sitosterol **(2)**, maritinone **(3)**, and 7–7′-biplumbagin **(4)**.*Cis*-isoshinanolone **(5)** and *trans*-isoshinanolone **(6)** were isolated from the ethyl acetate extract of the branches of *P. indica*. The structures of compounds **1-6** were characterized by analyses of their 1D and 2D NMR spectroscopic data, and finally confirmed by comparison with data in the literature. Plumbagin (**1**) is a major bioactive compound of *P. indica*, which possesses various biological activities, such as anticancer, antibacterial, antioxidant, antifungal, neuroprotective, hypolipidemic, and anti-inflammatory activities[19-20].

The structure of compound **3** was characterized by analyses of the $^{1}$H, $^{13}$C, DEPT (Distortionless Enhancement of Polarization Transfer) spectra, $^{1}$H-$^{1}$H COSY (Correlated spectroscopy), HSQC (Heteronuclear Single Quantum Coherence), and HMBC (Heteronuclear Multiple Bond Correlation) spectroscopic data (**Table 1**). The $^{1}$H-NMR spectrum of **3** demonstrated resonances of a hydroxy proton ($\delta_H$ 12.58), three aromatic protons ($\delta_H$ 7.29, 7.20, 6.81) and methyl protons ($\delta_H$ 2.01). Analyses of the $^{13}$C and DEPT of **3** revealed the presence of three methine, one methyl, and seven quaternary carbons. The HSQC spectrum helped to assign the attached protons to their corresponding carbon atoms, while the $^{1}$H-$^{1}$H COSY spectrum demonstrated the partial structure from H-6 to H-7. The HMBC spectra showed the correlations of the OH-5 to C-5, C-6, and C-10 and from the methyl protons H-11 to C-1, C-2, and C-3, thus establishing that the OH group was attached to C-5 and the methyl groups H-11 to C-2, respectively. The HMBC spectrum also showed the correlations of H-3 to C-1, C-10, and C-11; H-6 to C-5, C-8, and C-10; H-7 to C-5, C-8, and C-9. The splitting pattern for H-6 and H-7 was simplified due to the absence of H-8, which established the dimer linkage at C-8 to C-8′, suggesting that compound **3** was a dimer of compound **1**. The structure of compound **3** was identified as 8,8′-biplumbagin by spectroscopic methods (1D and 2D NMR) including a comparison with data reported in the literature[21] and was named maritinone (**Figure 1**).

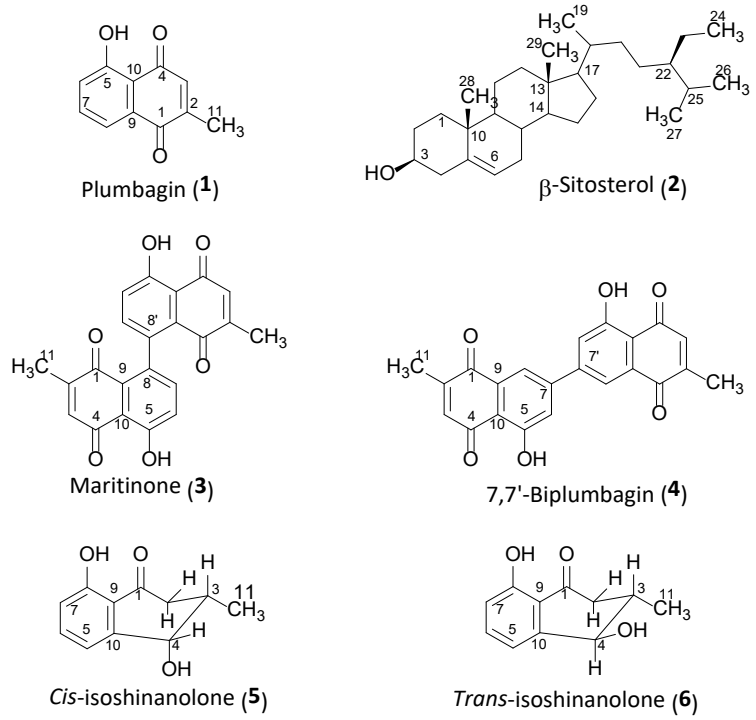



**Figure 1.** Structures of compounds **1–6**.

**Table 1.** $^{1}H$, $^{13}C$ and HMBC NMR spectroscopic data of compounds **1**, **3** and **4** in $CDCl_3$.

| Position | Compound **1** | | Compound **3** | | | Compound **4** | | |
|---|---|---|---|---|---|---|---|---|
| | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | HMBC | $\delta_H$ (mult., *J* in Hz) | $\delta_C$ | HMBC |
| 1 | - | 184.7 | - | 185.2 | - | - | 184.5 | - |
| 2 | - | 149.6 | - | 150.0 | - | - | 149.8 | - |
| 3 | 6.80, *q* (1.5) | 135.4 | 6.81, *q* (1.4) | 134.9 | C-1, C-10, C-11 | 6.85, *q* (1.3) | 135.5 | C-1, C-2, C-10, C-11 |
| 4 | - | 190.2 | - | 190.5 | - | - | 190.5 | - |
| 5-OH | 11.97, *s* | 161.1 | 12.58, *s* | 161.3 | C-5, C-6, C-10 | 12.5, *s* | 158.9 | C-5, C-6, C-10 |
| 6 | 7.25, *dd* (7.5, 1.4) | 124.1 | 7.29, *d* (8.7) | 124.3 | C-5, C-8, C-10 | 7.73, *s* | 118.7 | C-5, C-7, C-8, C-10 |
| 7 | 7.60, *dd* (8.1, 7.5) | 136.1 | 7.20, *d* (8.7) | 138.0 | C-5, C-8, C-9 | - | 131.2 | - |
| 8 | 7.63, *dd* (8.1, 1.4) | 119.2 | - | 135.6 | - | 7.73, *s* | 137.7 | C-1, C-6, C-7, C-10 |
| 9 | - | 132.0 | - | 128.2 | - | - | 131.9 | - |
| 10 | - | 115.1 | - | 115.5 | - | - | 115.3 | - |
| 11-$CH_3$ | 2.19, *d* (1.5) | 16.5 | 2.01, *d* (1.4) | 16.6 | C-1, C-2, C-3 | 2.22, *d* (1.3) | 16.5 | C-1, C-2, C-3 |

Compound **4** was obtained as a red solid. Like compound **1**, the $^{1}H$ and $^{13}C$ NMR spectra of compound **4** (**Table 1**) showed the characteristic signals of the plumbagin skeleton at $\delta_H$ 12.50 (1H, *s*, OH-5), 7.73 (2H, *s*, H-6 and H-8), 6.84 (1H, *q*, *J* = 1.4 Hz, H-3), and 2.22 (3H, *d*, *J* = 1.4 Hz, H-11); $\delta_C$ 190.5 (C-4), 184.5 (C-1), 158.9 (C-5), 149.8 (C-2), 137.7 (C-8), 135.5 (C-3), 131.9 (C-9), 131.2 (C-7), 118.7 (C-6), 115.3 (C-10), and 16.5 (C-11). The NMR comparison of compound **4** with compound **1** revealed that an aromatic proton, H-7, in compound **1** ($\delta_H$ 7.60) was absent. The doublet of doublet proton signals at H-6 and H-8 in compound **1** were replaced by singlet protons at H-6 and H-8 in compound **4**. Therefore, compound **4** was a dimer of plumbagin that showed a linkage between the 7 and 7′ positions. Analyses of $^{1}H$-$^{1}H$ COSY and HMBC spectra of compound **4**, combination with a comparison of data from the literature[22], led to the assignment of the proton and carbon resonances in compound **4**. The key HMBC correlations were as follows: H-3 to C-1, C-2, C-10 and C-11; OH-5 to C-5, C-6, and C-10; H-6 to C-5, C-7, C-8 and C-10; H-8 to C-1, C-6, C-7 and C-10; and H-11 to C-1, C-2 and C-3. The structure of compound **4** has thus been established in the present study as 7–7′-biplumbagin[22], the first time it has been isolated from *P. indica.*

Compound **5** (15.7 mg, 0.29%) was obtained as a colorless oil. The $^{1}H$ NMR spectrum (**Table 2**) showed ten resonances: two hydroxy protons [$\delta_H$ 1.98 (1H, *d*, *J* = 3.0 Hz, OH-4) and 12.40 (1H, *s*, OH-8)], three aromatic protons [$\delta_H$ 6.92 (1H, *dd*, *J* = 8.4 and 1.0 Hz, H-5), 7.47 (1H, *dd*, *J* = 8.3 and 7.5 Hz, H-6) and 6.91 (1H, *dd*, *J* = 7.4 and 1.0 Hz, H-7)], two methine protons [$\delta_H$ 4.73 (1H, *br d*, *J* = 2.4 Hz, H-4) and 2.42 (1H, *m*, H-3)]; methylene protons [$\delta_H$ 2.85 (1H, *dd*, *J* = 17.6 and 11.0 Hz, H-2a) and 2.55 (1H, *dd*, *J* = 17.6 and 4.3 Hz, H-2b)] and methyl protons ($\delta_H$ 1.16, 3H, *d*, *J* = 6.9 Hz, H-11). The $^{13}C$ NMR and DEPT spectral data revealed eleven carbon signals, characterized as: five methine carbons at $\delta_C$ 34.5 (C-3), 71.2 (C-4), 118.2 (C-7), 118.8 (C-5), 137.1 (C-6); a methylene carbon at $\delta_C$ 40.8 (C-2); a methyl

**Table 2.** $^{1}H$ and $^{13}C$ and HMBC NMR spectroscopic data ($\delta$ in ppm, $J$ in Hz) of compounds **5** and **6** in $CDCl_3$.

| Position | Compound **5** | | | Compound **6** | | |
|---|---|---|---|---|---|---|
| | $\delta_H$ ($J$ in Hz) | $\delta_C$ | HMBC | $\delta_H$ ($J$ in Hz) | $\delta_C$ | HMBC |
| 1 | - | 204.9 | - | - | 203.9 | - |
| 2 | 2.85, *dd* (11.0, 17.6)[a]<br>2.55, *dd* (17.6, 4.3)[b] | 40.8 | C-1, C-3, C-4, C-9, C-11 | 2.91, *dd* (4.1, 17.4)[a]<br>2.43, *dd* (17.4, 10.15)[b] | 43.5 | C-1, C-3, C-4, C-9, C-11 |
| 3 | 2.42, *m* | 34.5 | C-1, C-2, C-11 | 2.27, *m* | 37.6 | C-1, C-2, C-4, C-10, C-11 |
| 4 | 4.73, *br d* (2.4) | 71.2 | C-2, C-3, C-5, C-9, C-10, C-11 | 4.50, *br d* (7.4) | 73.8 | C-2, C-3, C-5, C-9, C-10, C-11 |
| 4-OH | 1.98, *d* (3.0) | - | C-3, C-4, C-10 | 2.18, *d* (6.5) | - | C-3, C-4, C-10 |
| 5 | 6.92, *dd* (8.4, 1.0) | 118.8 | C-4, C-6, C-7, C-9, C-10 | 7.11, *d* (7.5) | 117.4 | C-4, C-6, C-7, C-9, C-10 |
| 6 | 7.47, *dd* (7.5, 8.3) | 137.1 | C-4, C-5, C-7, C-8, C-10 | 7.50, *t* (8.0) | 137.2 | C-5, C-7, C-8, C-10 |
| 7 | 6.91, *dd* (7.4, 1.0) | 118.2 | C-5, C-6, C-8, C-9 | 6.90, *d* (8.3) | 117.3 | C-5, C-8, C-9 |
| 8-OH | 12.40, *s* | 162.8 | C-7, C-8, C-9 | 12.36, *s* | 162.6 | C-7, C-8, C-9 |
| 9 | - | 115.1 | C-2, C-4, C-3 | | 115.4 | - |
| 10 | - | 145.2 | - | - | 146.0 | - |
| 11-$CH_3$ | 1.16, *d* (6.9) | 16.3 | C-2, C-3, C-4 | 1.18, *d* (6.4) | 17.9 | C-2, C-3, C-4 |

[a,b] Interchangeable resonances.

carbon at $\delta_C$ 16.3 ($CH_3$-11); and four quaternary carbons at $\delta_C$ 204.9 (C-1), 162.8 (C-8), 145.2 (C-10) and 115.1 (C-9). The $^{1}H$-$^{1}H$ COSY spectrum revealed the proton-proton connectivity of H-2 to H-4, H-5 to H-7 and also the coupling between H-3 and H-11. The HMBC correlation established the connections of proton signals from H-2, H-7, and OH-8 to C-9; H-4, H-5, H-6, and OH-4 to C-10; and H-2 and H-3 to C-1, thus suggesting the naphthoquinone unit. The relative configurations at C-3 and C-4 of compound **5** can be proposed from the observed vicinal $^{1}H$-$^{1}H$ coupling constant ($J$ = 2.4 Hz) between H-3 and H-4, indicating an *axial-equatorial* orientation of these protons, thus supporting a 3,4-*cis* configuration. Therefore, compound **5** was identified as *cis*-isoshinanolone. It has been previously isolated from various plant families, for example, Iridaceae, Plumbaginaceae, Nepenthaceae, Dioncophyllaceae, Ancistrocladaceae, and Ebenaceae[23-24].

Compound **6** was obtained as a white solid. Comparison of the $^{1}H$ and $^{13}C$ NMR spectroscopic data with that of compound **5** revealed that compound **6** had a similar pattern except for the coupling constant ($J$ = 2.4 Hz) between H-3 and H-4 in compound **5** (**Table 2**). The large coupling constant ($J$ = 7.4 Hz) between H-3 and H-4 indicated 3,4-*trans* configuration. Consequently, compound **6** was identified as *trans*-isoshinanolone[23-24].

## Antifungal assay

The antifungal activities of compounds **1-3** and **5-6** were evaluated against *A. flavus* and *T. marneffei* (**Table 3**). Compounds **1**, **5** and **6**

exhibited zones of inhibition of 40.0, 7.0, and 7.0 mm (at 1.0 mg/disc), respectively, for *A. flavus*. Compounds **5** and **6** exhibited zones of inhibition of 12.0 and 10.0 mm (at 0.25 mg/disc), respectively, for *T. marneffei.* Compound **1** showed a remarkable level of activity against *T. marneffei*, exhibiting zones of inhibition of 67.5, 67.5, and 70.0 mm at 0.25, 0.5 and 1.0 mg/disc, respectively. The linkage between the 8 and 8′ positions of plumbagin in compound **3** led to a significant loss of antifungal activity. Compounds **5** and **6** exhibited similar antifungal activity, indicating that the *cis*- and *trans*-configurations between H-3 and H-4 were not critical for their antifungal activity.

**Table 3.** Antifungal activity of compounds **1-3** and **5-6.**

| Crude extracts or compounds | Diameter of inhibition zones (mm) | | | | | |
|---|---|---|---|---|---|---|
| | *A. flavus* | | | *T. marneffei* | | |
| | 0.25 mg/ disc | 0.5 mg/ disc | 1.0 mg/ disc | 0.25 mg/ disc | 0.5 mg/ disc | 1.0 mg/ disc |
| Crude $CH_2Cl_2$ | -[a] | - | 19.0 | - | - | 43.3 |
| Crude EtOAc | - | - | IN[b] | - | - | IN |
| 1 | 40.0 | 40.0 | 40.0 | 67.5 | 67.5 | 70.0 |
| 2 | IN | IN | IN | IN | IN | IN |
| 3 | IN | IN | IN | IN | IN | IN |
| 5 | IN | IN | 7.0 | 12.0 | 15.0 | 20.0 |
| 6 | IN | IN | 7.0 | 10.0 | 18.0 | 20.0 |

[a]-, not test. [b]IN, inactive.

## Conclusion

The present study has described the isolation and characterization of six constituents, plumbagin (**1**), *β*-sitosterol (**2**), maritinone **(3)**, 7–7′ -biplumbagin **(4)**, *cis*-isoshinanolone (**5**), and *trans*-isoshinanolone **(6)**, extracted from the branches of *P. indica*. This is the first report on the isolation of compounds **3, 4** and **6** from *P. indica*. The antifungal activity of some of the compounds was also evaluated, with compounds **1**, **5** and **6** displaying inhibition against *A. flavus* and *T. marneffei*, and compounds **2** and **3** exhibiting no antifungal activity. These results suggest that plumbagin can be considered the active chemical constituent in *P. indica* responsible for the antifungal activity against *A. flavus* and *T. marneffei.* This is the first study to report on the antifungal activity of compounds **1, 5** and **6** against *A. flavus* and *T. marneffei.*


## Acknowledgements

This work was supported by the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Ministry of Higher Education, Science, Research and Innovation and the Department of Chemistry, Faculty of Science, Srinakharinwirot University, Thailand. We are indebted to Chulabhorn Research Institute (Bangkok, Thailand) for recording the NMR spectroscopy and to the Bioassay Research Facility of the National Center for Genetic Engineering and Biotechnology (BIOTEC) (Pathum Thani, Thailand) for recording the high-resolution mass spectra. We thank Phillip Creed, PhD, from Edanz (http://www.edanz.com/ac) for editing a draft of this manuscript.


## References


1. Priyanjani HASA, Senarath RMUS, Senarath WTPSK, Munasinghe MLAMS. Propagation,

Phytochemistry and Pharmacology of *Plumbago* indica - A Review. *J. Pharm. Res. Int.,* 2021;33(42B):188-202.doi:10.9734/jpri/2021/v33i42B32439
2. Thitiorul S, Ratanavalachai T, Tanuchit S, Itharat A, Sakpakdeejaroen I. Genotoxicity and interference with cell cycle activities by an ethanolic extract from Thai *Plumbago indica* roots in human lymphocytes in vitro. *Asian Pac. J. of Cancer Prev.* 2013;14(4): 2487-2490. doi:10.7314/apjcp.2013.14.4.2487
3. Ittiyavirah SP, Jobin KV, Jissa MS, Jomy M, Josmi TJ, Littin B. Anti-inflammatory and analgesic activities of *Plumbago capensis* and *Plumbago indica. Adv. Pharmacol. Toxicol.* 2012;13(1): 47-52.
4. Rondeau G, Abedinpour P, Chrastina A, Pelayo J, Borgstrom P, Welsh J. Differential gene expression induced by anti-cancer agent plumbagin is mediated by androgen receptor in prostate cancer cells. *Sci. Rep.* 2018; 8(1):2694.doi:10.1038/s41598-018-20451-9
5. Cao Y, Yin X, Jia Y, Liu B, Wu S, Shang M. Plumbagin, a natural naphthoquinone, inhibits the growth of esophageal squamous cell carcinoma cells through inactivation of STAT3. *Int. J. Mol. Med.* 2018;42(3): 1569-1576. doi:10.3892/ijmm.2018.3722
6. Malaiyandia J, Anandapadmanabanb G, Perumalsamyc H, Kilankajaed A, Chellappanf DK, Duag K, et al. Plumbagin from two Plumbago species inhibits the growth of stomach and breast cancer cell lines. *Ind. Crops. Prod.* 2020;146: 112147.doi:10.1016/j.indcrop.2020.112147
7. Dissanayake DMIH, Perera DDBD, Keerthirathna LR, Heendeniya S, Anderson RJ, Williams DE, et al. Antimicrobial activity of Plumbago indica and ligand screening of plumbagin against methicillin-resistant *Staphylococcus aureus. J Biomol. Struct. Dyn.* 2022;40(7): 3273-3284. doi:10.1080/07391102.2020.1846622
8. Sil SK. A review on pharmacological profiles of *Plumbago indica L.* and *P. zeylanica L. Ind. J. Physiol. Allied Sci.* 2015;69(3): 83-89. doi:10.55184/ijpas.v69i03.13
9. Panyaphu K, Sirisa-ard P, Ubol PN, Nathakarnkitkul S, Chansakaow S, On TV. Phytochemical, antioxidant and antibacterial activities of medicinal plants used in Northern Thailand as postpartum herbal bath recipes by the Mien (Yao) community. *Phytopharmacology.* 2012;2(1): 92-105.
10. Eldhose B, Notario V, Latha MS. Evaluation of phytochemical constituents and In vitro antioxidant activities of *Plumbago indica* root extract. *J. Pharmacogn. Phytochem.* 2013;2(4): 157-161.
11. Ibrahim M, Baura J, Islam T, Homa Z, Chowdhury M, Hossain A, et al. Preliminary phytochemical and pharmacological investigations of *Alpinia conchigera* Griff. and *Plumbago indica L. Bangla. Pharma. J.* 2012;15(2):153-157.doi:10.3329/bpj.v15i2.12581
12. Yu B, Zhu X, Yang X, Jin L, Xu J, Ma T, et al. Plumbagin prevents secretory diarrhea by inhibiting CaCC and CFTR channel activities. *Front. Pharmacol.* 2019;10: 1181.doi:10.3389/fphar.2019.01181
13. Savadi RV, Alagawadi KR. Antifertility activity of ethanolic extracts of *Plumbago indica* and *Aerva lanata* on albino rats. *Int. J. Green Pharm.* 2009;3: 230-233.
14. Dasgupta GM, Thangamani D, Thapliyal M, Yasodha R, Gurumurthi K. Purification of a 20 KDa antifungal protein from *Plumbago capensis* a medicinal plant. *J. Med. Aromat. Plant Sci.* 2002;24: 16-18.
15. Saraswathy A, Chandran RVP, Manohar BM, Vairamuthu S. Wound healing activity of the chloroform extract of *Plumbago rosea Linn.* and Plumbagin. *Nat. Prod. Sci.* 2006;12(1): 50-54.
16. Sujin JKT, Balavigneswaran CK, Prakash S, Natheer HY, Srinivasakumar KP. Excision wound healing and antioxidant activity of different root extracts of *Plumbago indica. Med. Plant Res.* 2013;3(3): 13-19.doi: 10.5376/mpr.2013.03.0003
17. Rafa ZT, Ashrafudoulla M, Fuad F, Islam R, Hasan M, Kafi MAH, et al. Phytochemical and pharmacological investigation of *Plumbago indica L. J. Med. Plants Stud.* 2016;4(2): 115-118.

18. Bansod S, Rai M. Antifungal activity of essential oils from Indian medicinal plants against human pathogenic *Aspergillus fumigatus* and *A. niger. World J. Med. Sci.* 2008;3(2):81-88.
19. Liu Y, Cai Y, He C, Chen M, Li H. Anticancer properties and pharmaceutical applications of plumbagin: A review. *Am. J. Chin. Med.* 2017;45(3):423-441.doi:10.1142/0192415X-17500264
20. Guan HH, Huang YH, Lin ES, Chen CJ, Huang CY. Plumbagin, a natural product with potent anticancer activities, binds to and inhibits dihydroorotase, a key enzyme in pyrimidine biosynthesis. *Int. J. Mol. Sci.* 2021;22(13): 6861. doi:10.3390/ijms22136861
21. Gu JQ, Graf TN, Lee D, Chai HB, Mi Q, Kardono LBS, et al. Cytotoxic and antimicrobial constituents of the bark of *Diospyros maritima* Collected in two geographical locations in Indonesia. *J. Nat. Prod.*, 2004; 67(7): 1156-1161. doi:10.1021/np040027m
22. Salae AW, Karalai C, Ponglimanont C, Kanjana-Opas AK, Yuenyongsawad S. Naphthalene derivatives from *Diospyros wallichii. Can. J. Chem.* 2010;88: 922–927. doi:10.1139/V10-084
23. Bringmann G, Münchbach M, Messer K, Koppler D, Michel M, Schupp O, et al. *Cis*- and *trans*-isoshinanolone from *Dioncophyllum thollonii:* absolute configuration of two `known', wide-spread natural products. *Phytochemistry.* 1999; 51(5): 693-699. doi: 10.1016/S0031-9422(99)00080-1
24. Jetty A, Subhakar C, Rajagopal D, Jetty M, Subramanyam M, Murthy MM. Antimicrobial activities of neo- and 1-epineoisoshinanolones from *Plumbago zeylanica* roots. *Pharm. Biol.* 2010;48(9): 1007–1011. doi:10.3109/13880200903433760

## License, Supplymentary Material and Copyright

This is an open-access article distribute under the terms of the Creative Commons Attribution (CC by NC ND 4.0) License. You may share the material, but must give appropriate credit to the source, provide a link to the license and indicate if changes were mades. You may not use the material for commercial purpose. If you remix, tranform, or build upon the material, you may not distribute the modified material

Any supplymentary material reference in the article can be found in the online version.

This article is copyright of the Chulabhorn Royal Academy, 2022

## Citation

Manyaem S., Namsa-ard M., Puttikamonkul S., Prachya S., Namsa-ard A., Samosorn S. Chemical Constituents and their Antifungal Activity from the Branch Extracts of *Plumbago indica L. J Chulabhorn Royal Acad.* 2022; 4(4): 189-197 https://he02.tci-thaijo.org/index.php/jcra/article/view/258386

## Online Access

https://he02.tci-thaijo.org/index.php/jcra/article/view/258386