ISSN 1859 - 4735

JOURNAL OF MEDICINAL MATERIALS

No. 3 - Vol. 30
6/2025

NATIONAL INSTITUTE OF MEDICINAL MATERIALS

3B QUANG TRUNG - HOAN KIEM - HANOI - VIETNAM
TEL: (+84-24) 38247058 FAX: (+84-24) 39348740
Email: tapchiduoclieu@nimm.org.vn; tapchiduoclieu@gmail.com

JOURNAL OF MEDICINAL MATERIALS (Print and Electronic Journals)

EDITORIAL BOARD (Term 2025-2030)

(Attached to Decision No. 666/QD-VDL dated April 24, 2025 signed by the Director of Vietnam National Institute of Medicinal Materials)

EDITOR-IN-CHIEF

Assoc. Prof. PhD. Do Thi Ha

I. Subcommittee of Pharmacognosy - Traditional Medicine

- **Assoc. Prof. Dr. Sci. Nguyen Minh Khoi - Head of Subcommittee**
- Assoc. Prof. PhD. Nguyen Thuong Dong - Member
- Prof. PhD. Nguyen Minh Duc - Member
- Prof. PhD. Tran Cong Luan - Member
- Prof. PhD. Won Keun Oh - Member
- Assoc. Prof. PhD. Tran Hung - Member
- Assoc. Prof. PhD. Nguyen Thi Bich Thu - Member
- Assoc. Prof. PhD. Phuong Thien Thuong - Member
- Assoc. Prof. PhD. Phan Minh Giang - Member
- Assoc. Prof. PhD. Le Nguyen Thanh - Member
- Assoc. Prof. PhD. Le Viet Dung - Member
- Assoc. Prof. PhD. Nguyen Quoc Huy - Member
- Assoc. Prof. PhD. Nguyen Thu Hang - Member
- PhD. Tran Minh Ngoc - Member
- PhD. Nguyen Van Tai - Member
- Assoc. Prof. PhD. Le Dinh Chi - Member
- PhD. Nguyen Tuan Hiep - Member.

II. Subcommittee of Pharmacology - Clinical Pharmacy

- **Assoc. Prof. PhD. Pham Thi Nguyet Hang - Head of Subcommittee**
- Assoc. Prof. Dr.Sci. Do Trung Dam - Member
- Assoc. Prof. PhD. Nguyen Thi Thu Huong - Member
- Assoc. PhD. William R. Folk - Member
- Assoc. Prof. PhD. Pham Thi Van Anh - Member
- Assoc. Prof. PhD. Nguyen Thuy Duong - Member
- Assoc. Prof. PhD. Le Van Minh - Member
- PhD. Tran Thi Hien - Member.

III. Subcommittee of Agriculture - Biology

- **PhD. Nguyen Van Khiem - Head of Subcommittee**
- PhD. Phan Thuy Hien - Member
- Assoc. Prof. PhD. Nguyen Van Tap - Member
- Assoc. Prof. PhD. Pham Thanh Huyen - Member
- Prof. PhD. Tran The Bach - Member.

IV. Subcommittee of Secretariat Administration

- **Assoc. Prof. PhD. Le Nguyen Thanh - Head of Subcommittee**
- PhD. Le Thi Xoan - Academic Secretary
- BSc. Nguyen Thi Minh Loc - Administrative Secretary.

CONTENTS VOL. 30(3), 2025
(Pages 145 - 224)

Page	SCIENTIFIC RESEARCH

Journal of Medicinal Materials, 2025, Vol. 30, No.3 (pp. 157 - 162)

ANTIMICROBIAL MEGASTIGMANES AND NUCLEOSIDES ISOLATED FROM THE LEAVES OF *THESPESIA POPULNEA*

Nguyen Phuong Thao[1,*], Kieu Thi Phuong Linh[1], Duong Thu Trang[1], Pham Thanh Binh[1], Nguyen The Cuong[2], Nguyen Van Thanh[1]

[1]*Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam;*

[2]*Institute of Biology, VAST, Hanoi, Vietnam*

*Corresponding author: thaonp@ich.vast.vn

Received April 24th, 2025 Accepted May 23th, 2025

Summary

A phytochemical investigation of the ethanolic extract of *Thespesia populnea* (L.) Sol. ex Correa leaves led to the isolation of six compounds (**1–6**), using various chromatographic techniques. Their structures were elucidated as dihydrophaseic acid (**1**), icariside B_1 (**2**), icariside B_2 (**3**), 3*β*,5*α*,6*β*,9*β*-tetrahydroxymegastigman-7-ene (**4**), L-thymidine (**5**), and L-uridine (**6**) based on detailed analyses of 1D and 2D NMR, as well as mass spectrometric data, and by comparison with previously reported structures. The antimicrobial assays demonstrated that compound **1** selectively inhibited *Candida albicans* fungus with an MIC value of 64 *μ*g/mL, while **4** exhibited weak inhibition against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Bacillus cereus* bacterial strains with an MIC value of 128 *μ*g/mL. This is the first time megastigmane and nucleoside constituents from *T. populnea* have been reported.

Keywords: *Thespesia populnea; Malvaceae; Megastigmane; Nucleoside; Antimicrobial activity*.

1. Introduction

Thespesia is a small genus of the family Malvaceae, comprising 18 species primarily found in tropical and subtropical climates around the world, particularly in Africa, the Americas, Asia, and the islands of the Pacific Ocean [1],[2],[3]. However, the genus *Thespesia* includes only two species [*Thespesia populnea* (L.) Sol. ex Correa and *Thespesia lampas* (Cav.) Dalzell & A. Gibson] that are present from the north to the south of Vietnam [4]. Among these, *T. populnea* is widely distributed in Hawaii, California, Florida, Africa, the Caribbean, and Asia [5]. The various parts of this tree have been used for different purposes, including antifertility, antibacterial, anti-inflammatory, antioxidant, purgative, and hepatoprotective activities [1],[2],[3]. Previous phytochemical investigations of this species have afforded highly oxidized sesquiterpenes [5],[6],[7], flavonoids [8],[9], and other constituents [10],[11],[12]. In our ongoing search for bioactive metabolites from Vietnam's natural resources, a phytochemical investigation from the leaves of *T. populnea* was conducted, resulting in the isolation of six compounds, including four megastigmanes and two nucleoside derivatives (**1**–**6**, Fig. 1). Additionally, the antimicrobial activity of these isolated compounds against seven microbial strains was also evaluated.

2. Material and methods

2.1. Chemicals and equipment

The melting points were measured using a Thermo Scientific 1401 Mel-Temp 3.0 melting point apparatus. Optical rotations were determined with a JASCO P-2000 polarimeter. UV spectra were recorded on a Chirascan spectrometer. NMR spectra were recorded on a Bruker AVANCE NEO 600 FT-NMR spectrometer, with tetramethylsilane (TMS) as the internal standard. ESI-MS was performed on an Agilent 6530 Accurate-Mass spectrometer. MPLC was carried out using a Biotage-Isolera One system. Column chromatography (CC) was conducted on *silica gel*, Sephadex LH-20, and RP-18. Analytical thin-layer chromatography was performed on precoated *silica gel* 60 F_{254} and RP-18 F_{254S} plates and compounds were visualized by spraying with 10% H_2SO_4 in water and then heating for 1.5 to 2 minutes.

2.2. Plant material

The leaves of *Thespesia populnea* (L.) Sol. ex Correa were collected at Halong Bay, Quang Ninh Province, Vietnam, in September 2023. The species was identified by Dr. Nguyen The Cuong. A voucher herbarium specimen has been deposited at the Herbarium of the Institute of Chemistry (VAST, Hanoi, Vietnam) with the accession number NCCB03.

2.3. Extraction and isolation

The dried and ground leaves (1.35 kg) were extracted five times with 3.5 L of EtOH using ultrasonication for 20 minutes, followed by maceration for at least 12 hours. This process yielded 60.9 g of extract after the evaporation of the solvent. The EtOH extract was then suspended in H_2O and subsequently extracted with CH_2Cl_2 and EtOAc,

resulting in 16.8 g of CH_2Cl_2 subextract, 21.3 g of EtOAc subextract, and 19.5 g of residue after the evaporation of the aqueous layer.

For further separation, a portion (21.3 g) of the EtOAc subextract was subjected to flash chromatography using *silica gel* as the stationary phase and a gradient solvent going from 100% CH_2Cl_2 to 100% EtOAc in 30 min and then to 100% MeOH in another 25 min. The flow rate was set to 50 mL/min, and 24 fractions of 100 mL were collected. The fractions were combined according to their similarity based on TLC analysis, affording 10 fractions (E1−E10). Fraction E4 (1.18 g) was further separated using Sephadex LH-20 chromatography (100 × 2.5 cm) with acetone as the solvent, yielding 12 fractions that were subsequently combined into four fractions (E4.1−E4.4) based on the TLC results. Fraction E4.3 (159.4 mg) was processed using the same column, yielding 95 vials that were combined through TLC analysis to produce five fractions (E4.3a−E4.3f). Among these, fraction E4.3c (0.58 g) was subjected to passage over a Sephadex LH-20 column (MeOH-H_2O, 1:2, v/v), resulting in the isolation of compounds **2** (6.3 mg) and **6** (5.1 mg). When the same steps were repeated as above, subfraction E4.2f (115 mg) was purified multiple times using *silica gel* with EtOAc-MeOH (15:1, v/v) as the eluent, followed by RP-18 CC (MeOH-H_2O, 1:1, v/v) to give compounds **1** (5.6 mg) and **3** (3.5 mg). Additionally, fraction E6 (1.56 g) was subjected to Sephadex LH-20 chromatography (100 × 2.5 cm, acetone), resulting in 210 vials that were combined into five fractions (E6.1−E6.6) according to the results of the TLC analysis. Finally, subfraction E6.4 (306 mg) was purified repeatedly using *silica gel* with EtOAc-MeOH (10:1, v/v) as the eluent, followed by RP-18 CC (MeOH-H_2O, 1:1, v/v), yielding compounds **4** (5.2 mg) and **5** (4.5 mg).

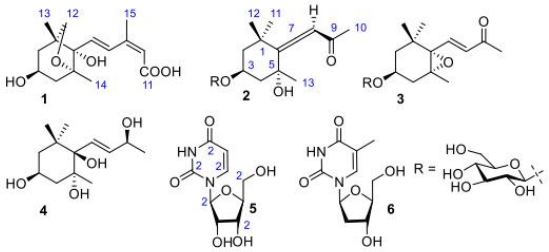

Fig. 1. Chemical structures of compounds **1**-**6**.

Dihydrophaseic acid (1): White, amorphous powder; $[\alpha]_D^{24}$ -29.7 (*c* 0.15, MeOH); ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectroscopic data, see Table 1; ESI-MS *m/z* 281.1 $[M - H]^-$.

Icariside B$_1$ (2): White, amorphous powder; $[\alpha]_D^{24}$ -85.3 (*c* 0.15, MeOH); UV (log ε) 232 (4.16) nm; ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectroscopic data, see Table 1; ESI-MS *m/z* 387.1 $[M + H]^+$.

Icariside B$_2$ (3): White, amorphous powder; mp. 172.5-174.0°C; $[\alpha]_D^{24}$ -115.8 (*c* 0.15, MeOH); UV (log ε) 230 (4.06) nm; ^{1}H (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz) spectroscopic data, see Table 1; ESI-MS *m/z* 385.1 $[M - H]^-$.

3β,5α,6β,9β-Tetrahydroxymegastigman-7-ene (4): White, amorphous powder; $[\alpha]_D^{24}$ -48.4 (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ_H: 1.47 (1H, ddd, *J* = 12.0, 4.0, 2.0 Hz, H_a-2), 1.66 (1H, t, *J* = 12.0 Hz, H_b-2), 4.08 (1H, m, H-3), 1.79 (1H, m, H_a-4), 1.75 (1H, t, *J* = 11.4 Hz, H_b-4), 6.08 (1H, dd, *J* = 16.2, 1.2 Hz, H-7), 5.80 (1H, dd, *J* = 16.2, 6.0 Hz, H-8), 4.36 (1H, dq, *J* = 6.0, 1.2 Hz, H-9), 1.27 (3H, d, *J* = 6.6 Hz, H-10), 1.21 (3H, s, H-11), 0.86 (3H, s, H-12), 1.15 (3H, s, H-13); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 40.6 (C-1), 46.5 (C-2), 65.2 (C-3), 45.7 (C-4), 77.8 (C-5), 78.9 (C-6), 131.2 (C-7), 136.1 (C-8), 69.5 (C-9), 24.2 (C-10), 27.5 (C-11), 26.2 (C-12), 27.1 (C-13); ESI-MS *m/z* 243.1 $[M - H]^-$.

L-uridine (5): White needles; mp. 160-161°C; $[\alpha]_D^{24}$-18.7 (*c* 0.55, H_2O); ^{1}H NMR (CD_3OD, 600 MHz) δ_H: 5.72 (1H, d, *J* = 8.5 Hz, H-5), 8.03 (1H, d, *J* = 8.5 Hz, H-6), 5.91 (1H, d, *J* = 5.0 Hz, H-1'), 4.20 (1H, t, *J* = 5.0 Hz, H-2'), 4.17 (1H, t, *J* = 5.0 Hz, H-3'), 4.02 (1H, m, H-4'), 3.75 (1H, dd, *J* = 12.0, 3.0 Hz, H_a-5'), 3.86 (1H, dd, *J* = 12.0, 5.0 Hz, H_b-5'); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 152.5 (C-2), 166.2 (C-4), 102.7 (C-5), 142.7 (C-6), 90.7 (C-1'), 75.7 (C-2'), 71.3 (C-3'), 86.4 (C-4'), 62.3 (C-5').

L-thymidine (6): White, amorphous powder; $[\alpha]_D^{24}$-48.9 (*c* 0.15, MeOH); ^{1}H NMR (CD_3OD, 600 MHz) δ_H: 7.83 (1H, br d, *J* = 1.2 Hz, H-6), 1.90 (3H, br d, *J* = 1.2 Hz, H-7), 6.29 (1H, t, *J* = 6.9 Hz, H-1'), 2.20-2.28 (2H, m, H-2'), 4.42 (1H, m, H-3'), 3.92 (1H, m, H-4'), 3.82 (1H, dd, *J* = 12.0, 3.0 Hz, H_a-5'), 3.75 (1H, dd, *J* = 12.0, 6.6 Hz, H_b-5'); ^{13}C NMR (CD_3OD, 150 MHz) δ_C: 152.4 (C-2), 166.4 (C-4), 111.5 (C-5), 138.2 (C-6), 12.4 (C-7), 86.2 (C-1'), 41.2 (C-2'), 72.2 (C-3'), 88.8 (C-4'), 62.8 (C-5').

2.4. In vitro antimicrobial assay

The antimicrobial activities of the extracts and isolated compounds were evaluated against seven bacterial strains: *Enterococcus faecalis* ATCC 299212, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 14579, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2753, and *Salmonella enterica* ATCC 13076, as well as one yeast, *Candida albicans* ATCC 10231. These

microorganisms were maintained on the agar slants in a refrigerator at 4°C. The strains were obtained from the American Type Culture Collection (ATCC). Mueller Hinton Broth (MHB) was used as the culture media for the *in vitro* antibacterial assay, while Luria Broth (LB) was used for *C. albicans*.

The minimum inhibitory concentration (MIC) values were determined using the broth microdilution method in conjunction with the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) colorimetric assay, as previously reported by J. M. Andrews [13]. Samples were dissolved in dimethyl sulfoxide (DMSO) at decreasing concentrations of 256, 128, 64, and 32 μg/mL with the number of experiments noted. The assay was carried out in 96-well plates. Each of these aliquots was then inoculated with 100 μL of suspension of the selected bacterial strains: *E. faecalis*, *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, *S. enterica*, and *C. albicans*. The test microbes were cultivated at 37°C for 24 hours and diluted according to the McFarland standard 0.5 scale. The inoculum prepared above was diluted in broth to give a final organism density of approximately 2×10^5 CFU/mL. The bioassay was carried out in flat-bottom 96-well microtiter plates. Ciprofloxacin (Sigma) and cycloheximide (Merck) were the positive controls for bacteria and yeast, respectively, while DMSO was used as a negative control. The number of colonies was counted in the first clear well displaying no visible growth (this is accomplished by spreading cultures out onto each appropriate agar plate). MIC was defined as the lowest concentration of samples that prevents visible growth of the microorganism in the microdilution wells, compared to that in the initial well (often expressed in μg/mL). The IC_{50} value was determined accurately based on BioTek spectrophotometer and Raw Data Software turbidity measurements. All experiments were done three times, in duplicates.

3. Results and discussion

Compound **1** was obtained as a white, amorphous powder. Its negative mode ESI-MS displayed a deprotonated molecular ion peak $[M - H]^-$ at *m/z* 281.1, corresponding to the molecular formula $C_{15}H_{22}O_5$, which indicates four degrees of unsaturation. The analysis of the $^1H/^{13}C$ NMR and HSQC spectra of **1** (Table 1) revealed signals indicative of two methyl groups [δ_H 0.95 (3H, s, H-13)/δ_C 16.3 (C-13) and 1.17 (3H, s, H-14)/δ_C 19.6 (C-14)], two methylene groups, one secondary oxymethine, one oxymethylene group, two oxygenated carbons, and one non-protonated carbon, suggesting the presence of an oxygen-bridged bicyclic ring skeleton. Additionally, a set of complex signals corresponding to one methyl group [δ_H 2.10 (3H, br s)/δ_C 21.2 (C-15)], two *trans*-olefinic proton signals [δ_H 6.53 (1H, d, J = 15.6 Hz, H-7)/δ_C 135.0 (C-7) and 7.98 (1H, d, J = 15.6 Hz, H-8)/δ_C 131.8 (C-8)], and other olefinic signals of one trisubstituted double bond [δ_H 5.79 (1H, br s, H-10)/δ_C 119.5 (C-10) and 151.2 (C-9)] indicated the presence of a 9-methyl-penta-7,9-dienoic acid moiety [14]. Confirmation of the partial structural units and their connectivity was established by the cross-peaks in the HMBC spectrum that were observed between H-7 (δ_H 6.53) with C-6 (δ_C 83.2); H-12 (δ_H 3.82/3.72) with C-1 (δ_C 49.4), C-5 (δ_C 87.8), and C-6; and H-14 (δ_H 1.17) with C-4 (δ_C 46.0), C-5, and C-6 (Fig. 2). Also, this conclusion was further supported by the downfield shift of C-6 in **1** (Table 1). These findings confirm the presence of a 9-methyl-penta-7,9-dienoic acid moiety linked to the ring at C-6. Based on the above evidence and comparisons with reported literature [14], compound **1** was finally concluded to be dihydrophaseic acid (Fig. 1). This compound has previously been obtained from the whole plants of *Gynostemma pentaphyllum* [14] and the seeds of *Phaseolus lunatus* [15]. However, to date, no studies have reported the biological activities of compound **1**.

Compound **2** was purified as a white, amorphous powder, with a negative optical rotation of $[\alpha]_D^{24}$ -85.3 (*c* 0.15, MeOH). The ^1H and ^{13}C NMR spectra of **2** revealed 19 carbon signals, 13 of which were attributed to a megastigmane skeleton. This included four tertiary methyl groups [δ_H 2.21 (3H, s, H-10)/δ_C 26.5 (C-10), 1.18 (3H, s, H-11)/δ_C 32.2 (C-11), 1.40 (3H, s, H-12)/δ_C 29.4 (C-12), and 1.41 (3H, s, H-13)/δ_C 30.8 (C-13)], one sp^3 non-protonated carbon [δ_C 37.0 (C-1)], one oxygenated carbon [δ_C 72.4 (C-5)], one oxymethine [δ_H 4.37 (1H, m, H-3)/δ_C 72.5 (C-3)], and two methylene groups [δ_H 2.11 (1H, ddd, J = 12.0, 4.2, 2.4 Hz, H_a-2)/1.48 (1H, dd, J = 12.0, 10.4 Hz, H_b-2)/δ_C 48.1 (C-2) and 2.05 (1H, dd, J = 13.8, 7.2 Hz, H_a-4)/1.75 (1H, dd, J = 13.8, 10.2 Hz, H_b-4)/δ_C 46.6 (C-4)]. Additionally, one *α,β*-unsaturated carbonyl [δ_C 200.9 (C-9)] and one allenic unit [δ_H 5.85 (1H, s, H-8)/δ_C 101.2 (C-8), 211.5 (C-7), and 120.1 (C-6)] were identified. Signals from a monosaccharide moiety were also observed (Table 1). An anomeric proton signal [δ_H 4.45 (1H, d, J = 7.8 Hz, H-1')] alongside signals attributed to other hydrogens of the sugar moiety (δ_H 3.88-3.17) were detected in the ^1H NMR spectrum. The sequential 1,2-*trans*-diaxial coupling patterns of H-1', H-2', H-3', H-4', and H-5' confirmed that the

hexose moiety was β-glucopyranoside (Table 1). The monoglucosylation position of **2** was reasonably assigned to C-3 rather than C-5, based on the downfield shift of one tertiary oxygenated carbon [δ_C

Table 1. NMR spectroscopic data for **1–3** and the reference compounds.

Pos.	$^{\#}\delta_C{}^a$	**1**		$^{\$}\delta_C{}^a$	**2**		$^{@}\delta_C{}^a$	**3**	
		$\delta_C{}^{a,b}$	$\delta_H{}^{a,c}$ mult. (J in Hz)		$\delta_C{}^{a,b}$	$\delta_H{}^{a,c}$ mult. (J in Hz)		$\delta_C{}^{a,b}$	$\delta_H{}^{a,c}$ mult. (J in Hz)
1	49.4	49.4	-	37.0	37.0	-	36.0	36.0	-
2	44.5	44.6	1.88 ddd (13.8, 7.2, 1.8) 1.68 dd (13.8, 10.4)	48.1	48.1	2.11 ddd (12.0, 4.2, 2.4) 1.48 dd (12.0, 10.4)	45.0	45.2	1.75 ddd (13.2, 3.0, 1.8) 1.43 dd (13.2, 10.2)
3	66.1	66.0	4.13 m	72.5	72.5	4.37 m	72.5	72.7	3.93 m
4	46.0	46.0	2.05 dd (13.8, 7.2) 1.75 dd (13.8, 10.2)	46.6	46.6	2.39 ddd (13.0, 4.2, 2.4) 1.46 dd (13.0, 11.4)	38.0	38.1	2.42 ddd (15.0, 4.8, 1.8) 1.83 dd (15.0, 8.4)
5	87.9	87.8	-	72.4	72.4	-	68.3	68.3	-
6	83.4	83.2	-	120.0	120.1	-	71.0	71.1	-
7	135.1	135.0	6.53 d (15.6)	211.5	211.5	-	145.2	145.2	7.18 d (15.6)
8	132.2	131.8	7.98 d (15.6)	101.2	101.2	5.85 s	133.6	133.8	6.20 d (15.6)
9	151.0	151.2	-	200.9	200.9	-	200.2	200.2	-
10	120.3	119.5	5.79 br s	26.5	26.5	2.21 s	27.3	27.4	2.31 s
11	170.6	170.4	-	32.2	32.2	1.18 s	29.3	29.4	1.23 s
12	77.4	77.3	3.82 d (7.2) 3.72 d (7.2)	29.4	29.4	1.40 s	25.3	25.5	0.98 s
13	16.2	16.3	0.95 s	30.8	30.8	1.41 s	20.0	20.2	1.21 s
14	19.5	19.6	1.17 s						
15	21.1	21.2	2.10 br s						
1'				102.6	102.7	4.45 d (7.8)	102.8	102.9	4.36 d (7.8)
2'				75.1	75.1	3.17 dd (9.0, 7.8)	74.9	75.1	3.14 dd (9.0, 7.8)
3'				78.1	78.1	3.38 dd (9.0, 9.0)	78.0	78.1	3.33 dd (9.0, 9.0)
4'				71.6	71.6	3.33 t (9.0)	71.4	71.6	3.29 dd (9.0, 8.4)
5'				77.9	77.9	3.35 m	77.7	77.9	3.27 m
6'				62.7	62.7	3.88 dd (12.0, 1.8) 3.70 dd (12.0, 4.8)	62.5	62.7	3.87 dd (12.0, 2.4) 3.68 dd (12.0, 5.4)

Measured in $^{a}CD_3OD$, ^{b}125 MHz, and ^{c}600 MHz. $^{\#}\delta_C$ of 4'-dihydrophaseic acid [14], $^{\$}\delta_C$ of icariside B_1 [16], and $^{@}\delta_C$ of icariside B_2 [17].

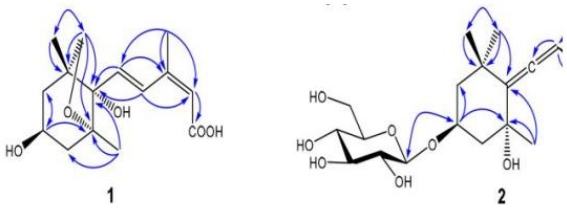

Fig. 2. Key HMBC correlations of **1** and **2**.

72.5 (C-3)] [16]. Furthermore, the attachment of the sugar moiety at C-3 was determined by the HMBC correlations between H-1' (δ_H 4.45) with C-3 (δ_C 72.5), and between H-3 (δ_H 4.37) with C-1' (δ_C 102.7) (Fig. 2). A comprehensive investigation of the NMR spectroscopic data and comparison with those from the published literature [16] supported the identification of **2** to be icariside B_1 (Fig. 1). To date, icariside B_1 has been widely recognized within the plant kingdom and has recently been isolated from numerous species across various plant families.

Compound **3** was obtained as a white, amorphous powder. The ^{1}H and ^{13}C NMR spectroscopic data suggested that compound **3** was a megastigmane glycoside [17]. Six signals were assignable to a β-glucopyranose moiety (Table 1). The anomeric proton of H-1' [δ_H 4.36 (1H, d, J = 7.8 Hz)] suggested that a sugar moiety is bound to the aglycone via a β-glucosidic linkage. The remaining 13 carbon signals comprised those of four methyl groups [δ_C 27.4 (C-10), 29.4 (C-11), 25.5 (C-12), and 20.2 (C-13)], two methylene groups [δ_C 45.2 (C-2) and 38.1 (C-4)], one 5,6-epoxy functionality [δ_C 68.3 (C-5) and 71.1 (C-6)], one oxymethine carbon [δ_C 72.7 (C-3)], one α,β-unsaturated carbonyl group [δ_C 200.2 (C-9)], along with a pair of *trans*-olefinic methine carbons [δ_C 145.2 (C-7) and 133.8 (C-8)] (Table 1). The disubstituted double bond is located between C-7 and C-8, while the ketone functional group was

part of a conjugated C-9 position. This conclusion is supported by the HMBC spectrum, which demonstrated that H_3-10 (δ_H 2.31) correlated with C-9 (δ_C 200.2). Furthermore, the position of the glucosidic linkage was determined to be at C-3 of the megastigmane core, as indicated by the HMBC spectrum, where H-1' (δ_H 4.36) correlated with the carbon signal at C-3 (δ_C 72.7). From the above evidence, compound **3** has been identified as icariside B_2 [17]. Thus far, no bioassays have been reported for this compound.

Compound **4** was purified as a white, amorphous powder. Its molecular formula was determined to be $C_{13}H_{24}O_4$ by the ESI-MS ion peak at *m/z* 243.1 $[M - H]^-$. The ^1H and ^{13}C NMR data of **4** revealed 13 carbon signals, ascribable to four methyl groups, two methylenes, one sp^3 non-protonated carbon, two oxygenated carbons, two oxymethines, along with a pair of *trans*-olefinic methine carbons. The double bond accounted for one degree of unsaturation, implying the presence of a ring in **4** to explain the second degree of unsaturation. Additionally, the large coupling constant between H-7 and H-8 (J = 16.2 Hz) indicated the *E*-configuration of the Δ^7 double bond. The above NMR spectroscopic data suggested that compound **4** possesses a megastigm-7-ene skeleton with four hydroxy groups assigned at C-3, C-5, C-6, and C-9. Careful comparison of the NMR spectroscopic data of **4** was similar to that of 3*β*,5*α*,6*β*,9*β*-tetrahydroxymegastigman-7-ene [18]. Besides, the negative sign of the specific rotation of **4** ($[\alpha]_D^{24}$ -48.4) was found to be identical to that of 3*β*,5*α*,6*β*,9*β*-tetrahydroxymegastigman-7-ene [18]. All of these data **4** were also consistent with the above deductions and comparable to each other. Consequently, the structure of **4** was fully established as 3*β*,5*α*,6*β*,9*β*-tetrahydroxymegastigman-7-ene (Fig. 1).

Two remaining compounds were determined to be L-uridine (**5**) [19] and L-thymidine (**6**) [19] by comparison of their physical and spectroscopic data to those reported in the references.

Considering the traditional uses of *T. populnea*, all isolated compounds were evaluated for their *in vitro* antimicrobial activity. The antibacterial assays were conducted against both Gram-positive bacteria (*E. faecalis*, *S. aureus*, and *B. cereus*) and Gram-negative bacteria (*E. coli*, *P. aeruginosa*, and *S. enterica*). Additionally, the antifungal activity was assessed against *C. albicans* using a previously reported method [13]. Ciprofloxacin and cyclohexamide were used as reference compounds. Among all the tested compounds, only compound **1** selectively inhibited antifungal activity against *C. albicans* with an MIC value of 64 μg/mL. Compound **4** exhibited weak inhibition against the Gram-positive strains *E. faecalis*, *S. aureus*, and *B. cereus* with an MIC value of 128 μg/mL. However, this compound was nearly ineffective against the Gram-negative bacteria.

The investigation of the antimicrobial properties of magastigmane constituents found that plantaginoside, (6*S*,9*R*)-7,8-dihydroroseoside, and roseoside from the flowers of Hosta plantaginea exhibited bacteriostatic effects against four microbial strains *B. cereus*, *B. aureus*, *E. coli*, and *Y. enterocolitica*, with the MIC values ranging from 28.4 to 276.2 μg/mL [20]. Huong et al. recently isolated (6*S*,7*E*,9*R*)-6,9-dihydroxy-4,7-megastigmadien-3-one 9-*O*-[*α*-L-arabinopyranosyl-(1→6)-*β*-D-glucopyranoside] and blumenol A from the leaves of the mangrove plant *Rhizophora stylosa* and examined their effect against *A. niger* and *F. oxysporum* strains, which exhibited moderate inhibition with MIC values of 50 and 100 µg/mL, respectively [21]. Similarly, Wang et al. reported that the antibacterial activity of austroside A was tested against an antibiotic-resistant strain of *S. aureus*. At equimolar concentrations, it produced inhibition zone diameters of 9 mm (190 µg/disc) [22]. Additionally, (3*R*,5*R*,6*S*,7*E*)-5,6-epoxy-3-hydroxy-megastigman-7-en-9-one 3-*O*-neohesperidoside and byzantionoside B were screened against four pathogens: *Pseudomonas aeruginosa*, *P. mirabilis*, *S. aureus*, and *C. albicans*.

When applied at a concentration of 30 µg/mL, a 20% inhibition of growth was observed after 24 hours [23].

Although some of the remaining compounds demonstrated relatively weak or negligible inhibitory activity, they still deserved our attention to further disclose their potential biological activities due to their similar derivatives with considerable related biological activities. In future research, it will be necessary to enhance the number of secondary metabolites produced by *T. populnea* to conduct a more comprehensive biological study.

4. Conclusion

The chemical constituents of *T. populnea* leaves have been identified in the present study. Six compounds were isolated, including dihydrophaseic acid (**1**), icariside B_1 (**2**), icariside

B_2 (**3**), 3*β*,5*α*,6*β*,9*β*-tetrahydroxymegastigman-7-ene (**4**), L-thymidine (**5**), and L-uridine (**6**). The structures of these compounds were elucidated through NMR spectroscopic analysis and comparison with previously reported data. To the best of our knowledge, the occurrence of compounds **1-6** has been reported for the first time in this plant. Among these, the megastigmane constituents are the predominant compounds. Furthermore, antimicrobial assays demonstrated that compounds **1** and **4** exhibit inhibitory effects against *E. faecalis*, *S. aureus*, *B. cereus*, and *C. albicans*. The findings of this study deepen the understanding of the chemical diversity present in *T. populnea* and may serve as a valuable reference for the development of antimicrobial medications derived from natural sources.

Acknowledgments: *This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2021.02.*

References

1. Suvarna C. H. M., Sriya P., Arshad M. D., Pavan K. (2018), A review on phytochemical and pharmacological properties of *Thespesia populnea. Journal of Drug Delivery and Therapeutics*, 8(4), 1-4.

2. Chumbhale D. S., Pawase A. A., Chaudhari S. R., Upasani C. D. (2018), Phytochemical, pharmacological and phytopharmaceutics aspects of *Thespesia populnea* (Linn.) Soland: a review. *Inventi Rapid: Ethnopharmacology*, 1(2), 1-5.

3. Reddy M. V. B. (2017), Review on chemistry, biological activity and biosynthesis of naturally occurring compounds of cadalene type sesquiterpenoids from Malvaceae. *Der Pharma Chemica*, 9, 40-49.

4. Xuyen D. T. (2017), Malvaceae Juss family. *The flora of Vietnam*, Publishing House for Science, 202-205.

5. Milbrodt M., Koenig W. A., Hausen B. M. (1997), 7-Hydroxy-2,3,5,6-tetrahydro-3,6,9-trimethylnaphtho[1,8-b,c]pyran-4,8-dione from *Thespesia populnea*. *Phytochemistry*, 45(7), 1523-1525.

6. Neelakantan S., Rajagopalan V., Raman P. V. (1983), Thespesone & thespone, two new mansonones of heartwood of *Thespesia populnea* Sol. ex Corr. (Fam. Malvaceae). *Indian Journal of Chemistry. Section B: Organic including Medicinal*, 22B, 95-96.

7. Li M. Y., Tian Y., Shen L., Buettner R., Li H. Z., Liu L., Yuan Y. C., Xiao Q., Wu J., Jove R. (2013), 3-*O*-Methylthespesilactam, a new small-molecule anticancer pan-JAK inhibitor against A2058 human melanoma cells. *Biochemical Pharmacology*, 86(10), 1411-1418.

8. Shirwaikar A., Srinivasan K. K. (1996), New flavonoids from the flowers of *Thespesia populnea. Journal of Medicinal and Aromatic Plant Sciences*, 18(2), 266-269.

9. Padumadasa C., Abeysekara A. M., Thabrew I., Ediriweera A. P. G. R. (2016), Pharmacological overview of proanthocyanidins from the bark of *Thespesia populnea* (L.) as an antioxidant and cytotoxic agent. *International Journal of Pharma Sciences and Research*, 7(1), 85-92.

10. Goyal M. M., Rani K. K. (1987), Chemical components from the leaves of *Thespesia populnea. Bangladesh Journal of Scientific and Industrial Research*, 22, 8-11.

11. Goyal M. M., Rani K. K. (1989), Antibacterial activity of the natural products from the leaves of *Thespesia populnea. Acta Ciencia Indica (Chemistry)*, 15, 117-124.

12. Goyal M. M., Rani K. K. (1985), *Thespesia populnea* - a rich source of *β*-sitosterol and lupeol. *Acta Ciencia Indica (Chemistry)*, 11, 163-164.

13. Andrews J. M. (2001), Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, 48(1), 5-16.

14. Zhang Z., Zhang W., Ji Y. P., Zhao Y., Wang C. G., Hu J. F. (2010), Gynostemosides A-E, megastigmane glycosides from *Gynostemma pentaphyllum. Phytochemistry*, 71(5-6), 693-700.

15. Li K., Bian J. H., Wua H., Wanga Y. I., Feng Y. J., Nie J., Na Chen, Yu H., Li W., Tan Y. Z., Lu G. H. (2024), Anti-inflammatory activity of organic acids from the seeds of *Phaseolus lunatus* L. *Phytochemistry Letters*, 61, 120-124.

16. Hisamoto M., Kikuzaki H., Nakatani N. (2004), Constituents of the leaves of *Peucedanum japonicum* Thunb. and their biological activity. *Journal of Agricultural and Food Chemistry*, 52(3), 445-450.

17. Feistel F., Paetz C., Lorenz S., Beran F., Kunert G., Schneider B. (2017), *Idesia polycarpa* (Salicaceae) leaf constituents and their toxic effect on *Cerura vinula* and *Lymantria dispar* (Lepidoptera) larvae. *Phytochemistry*, 143, 170-179.

18. Takeda Y., Okada Y., Masuda T., Hirata E., Shinzato T., Takushi A., Yu Q., Otsuka H. (2000), New megastigmane and tetraketide from the leaves of *Euscaphis japonica. Chemical and Pharmaceutical Bulletin*, 48(5), 752-754.

19. Moyroud E., Strazewski P. (1999), L-Ribonucleosides from L-xylose. *Tetrahedron*, 55(5), 1277-1284.

20. Bao X. H., Wang Q. H., Bao B. Y., Han J. J., Ao W. L. (2017), Antibacterial and antioxidant activities of megastigmane glycosides from *Hosta plantaginea. Chemistry of Natural Compounds*, 53(4), 614-617.

21. Huong P. T. T., Thanh N. V., Diep C. N., Thao N. P., Cuong N. T., Cuong N. X., Nam N. H., Minh C. V. (2015), Antimicrobial compounds from *Rhizophora stylosa. Vietnam Journal of Science and Technology*, 53(2), 205-210.

22. Wang J., Shen Y., He H., Kang W., Hao X. (2005), Norsesquiterpenoid and sesquiterpenoid glycosides from *Evodia austrosinensis. Planta Medica*, 71(1), 96-98.

23. Scognamiglio M., Buommino E., Coretti L., Graziani V., Russo R., Caputo P., Donnarumma G., D'Abrosca B., Fiorentino A. (2016), Phytochemical investigation and antimicrobial assessment of *Bellis sylvestris* leaves. *Phytochemistry Letters*, 17, 6-13.