STUDY ON CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF CONIFER SPECIES: PINUS DALATENSIS, PINUS KESIYA AND PODOCARPUS NERIIFOLIUS IN VIETNAM

Major: Organic Chemistry
Code: 62 44 01 14

SUMMARY OF CHEMISTRY DOCTORAL THESIS

Hanoi – 2017
Thesis was completed at:
Institute of Chemistry
Vietnam Academy of Science & Technology

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The dissertation will be defended at: Graduate University Science and Technology - Vietnam Academy of Science & Technology- No. 18 Hoang Quoc Viet - Cau Giay - Hanoi.
At............hour............day............month......... 2017
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INTRODUCTION

1. The urgency of the thesis

Nowadays, along with the rapid development of society, human are resisting many of the world's serious problems. Among the eight Millennium Development Goals (MDGs), goals of health are the top priorities. Climate change, environmental pollution and food contamination have been concluded as the causes of negative effects on human health as well as all living organisms on Earth. Developing drugs and treatments for new diseases and health-related problems have turned to be the most challenging and difficult tasks ever. Fortunately, one of the pathways to detect potential and effective compounds is studying on natural products. Lead compounds from various natural sources have been a basis for the development of important agents against various diseases.

Vietnam is located in tropical monsoon climate zone, so the country’s vegetation is rich and diversified (approximately 13766 different kinds of plants). In its natural vegetation ecology, conifer species have an important role in economic, trade, and culture. Many species in the genus Pinus and Podocarpus are used in traditional medicine for treatment of various diseases. Until now (2017), a lot of research on chemical constituents and biological activities of conifer species (about over 100 Pinus sp. and 80 Podocarpus sp.) have been published. However, there are still some other species in the two genera have not been researched. Among them, Pinus dalatensis has not been investigated previously, while researches on Pinus kesiya and Podocarpus neriifolius have just been in their initial and early stages. Therefore, it is practical and potential to investigate into the phytochemistry and biological activities of these species. The results of this study are also aimed to orient the research, exploitation and use of these plants in therapy.

2. The objectives of the thesis

Study on the chemical constituents and biological activities of some conifer species

3. The main contents of the thesis

Study on the chemical constituents of three conifers: Pinus dalatensis Pinus kesiya and Podocarpus neriifolius.
Some isolated compounds were tested biological activities in order to discover potential compounds.

Chapter 1. OVERVIEW

1.1. Introduction of the study species

1.1.1. Pinus dalatensis
1.1.2. Pinus kesiya
1.1.3. Podocarpus neriifolius

1.2. Study on the chemical constituents of some Pinus species

1.2.1. Study on the composition of essential oils from genus Pinus
1.2.2. Terpenoids from Pinus species
1.2.3. Flavonoids from Pinus species
1.2.4. Lignan from Pinus species
1.2.5. Other compounds from Pinus species

1.3. Study on biological activities of isolated compounds from Pinus species

1.3.1. Anti-inflammatory and analgesic activities
1.3.2. Antitumor and anticancer activities
1.3.3. Antibacterial and antifungal activities
1.3.4. Antioxidant activity.
1.3.5. Antiviral and other activities

1.4. Study on the chemical constituents and biological activities of some Podocarpus species

1.5. Study on the chemical constituents of Podocarpus neriifolius

Chapter 2. EXPERIMENT

This chaper describes the collection, determination, extraction, isolation, spectral data, and bioactivity assays of isolated compounds from these plant materials.
Spectral data of isolated compounds

**Compound TT1 (PDLE6): Caryolane-1β,9β-diol.**

Yellowish oil, 12 mg, \([\alpha]_D = + 0.7 \text{ (CHCl}_3\)]

$^1$H-NMR (CDCl$_3$, 500 MHz): $\delta_H$ 3.44 (1H, t, $J = 3.5 \text{ Hz}$, H-9), 2.19-2.23 (1H, m, H-2), 1.89 (1H, m, H-5) 1.19 (3H, brs, H-15), 1.02 (3H, brs, H-14), 1.00 (3H, brs, H-13). $^{13}$C-NMR (CDCl$_3$, 125 MHz): $\delta_C$ 70.7 (C-1), 38.2 (C-2), 34.1 (C-3), 35.1 (C-4), 44.0 (C-5), 20.5 (C-6), 35.5 (C-7), 39.4 (C-8), 72.3 (C-9), 28.2 (C-10), 33.5 (C-11), 42.5 (C-12), 20.8 (C-13), 30.6 (C-14), 26.6 (C-15).

**Mixture TT2 (PDLN3 và PDLE5):** the mixture of 16-Hydroxy-8(17),13-labdadien-15,16-olid-19-oic acid (TN2a) and 15-Hydroxypinusolidic acid (TN2b).

$^1$H-NMR (CDCl$_3$, 500 MHz): **TT2a** $\delta_H$ 5.97 (1H, brs, H-16), 5.84 (1H, brs, H-14), 4.89 (1H, brs, H-17a), 4.50 (1H, brs, H-17b), 1.24 (1H, s, H-18), 0.60 (1H, s, H-20). **TT2b** $\delta_H$ 6.82 (1H, brs, H-14), 6.10 (1H, brs, H-15), 4.89 (1H, brs, H-17a), 4.56 (1H, brs, H-17b), 1.24 (1H, s, H-18), 0.61 (1H, s, H-20). $^{13}$C-NMR (CDCl$_3$, 125 MHz): **TT2a** $\delta_C$ 39.2 (C-1), 21.1 (C-2), 37.9 (C-3), 44.2 (C-4), 56.2 (C-5), 26.0 (C-6), 38.6 (C-7), 147.3 (C-8), 55.6 (C-9), 40.6 (C-10), 21.8 (C-11), 26.8 (C-12), 171.5 (C-13), 117.1 (C-14), 171.5 (C-15), 99.2 (C-16), 106.8 (C-17), 29.0 (C-18), 183.0 (C-19), 12.9 (C-20). **TT2b** $\delta_C$ 39.2 (C-1), 19.9 (C-2), 37.9 (C-3), 44.2 (C-4), 56.2 (C-5), 26.0 (C-6), 38.0 (C-7), 147.4 (C-8), 55.7 (C-9), 40.5 (C-10), 21.8 (C-11), 24.3 (C-12), 138.6 (C-13), 143.3 (C-14), 97.3 (C-15), 171.9 (C-16), 106.8 (C-17), 29.0 (C-18), 183.0 (C-19), 12.9 (C-20).

**Compound TT3 (PDLE3): 15-Methoxypinusolic acid**

Yellowish oil, 6 mg, $[\alpha]_D = + 98.0 \text{ (CHCl}_3\)]

$^1$H-NMR (CDCl$_3$, 500 MHz): $\delta_H$ 6.77 (1H, s, H-14), 5.73 (1H, d, $J = 2.0 \text{ Hz}$, H-15), 4.89 (1H, s, H-17a), 4.57 (1H, d, $J = 6.0 \text{ Hz}$, H-17b), 3.57 (3H, brs, 15-OCH$_3$), 1.24 (3H, brs, H-18), 0.60 (3H, brs, H-20). $^{13}$C-NMR (CDCl$_3$, 125 MHz): $\delta_C$ 39.2 (C-1), 19.9 (C-2), 38.0 (C-3), 44.1 (C-4), 56.2 (C-5), 26.0 (C-6), 38.6 (C-7), 147.2 (C-8), 55.7 (C-9), 40.5 (C-10), 21.8 (C-11), 24.6 (C-12), 139.2 (C-13), 141.5 (C-14), 102.5 (C-15), 171.4 (C-16), 106.8 (C-17), 29.0 (C-18), 182.5 (C-19), 12.8 (C-20), 57.0 (15-OCH$_3$).
❖ **Compound TT4 (PDWE10): Lambertianic acid (155)**

Colorless solid, 35 mg, \([\alpha]_D = +80.0\) (MeOH), mp 127 °C, ESI-MS: \(m/z\) 315.2 [M-H]+, 339.3 [M+Na]+, molecular formula: \(C_{20}H_{28}O_{3}\)

\(\text{\(^1H-NMR\) (CDCl\(_3\), 500 MHz):}\) \(\delta_H\) 7.34 (1H, \(m\), H-15), 7.19 (1H, \(brs\), H-16), 6.25 (1H, \(d\), \(J= 0.5\) Hz, H-14), 4.88 (1H, \(s\), H-17a), 4.57 (1H, \(s\), H-17b), 1.23 (3H, \(brs\), H-18), 0.60 (3H, \(brs\), H-20). \(\text{\(^{13}C-NMR\) (CDCl\(_3\), 125 MHz):}\) \(\delta_C\) 39.0 (C-1), 19.9 (C-2), 37.8 (C-3), 44.2 (C-4), 56.3 (C-5), 26.0 (C-6), 38.7 (C-7), 147.9 (C-8), 55.2 (C-9), 40.4 (C-10), 23.6 (C-11), 24.3 (C-12), 125.4 (C-13), 110.9 (C-14), 142.7 (C-15), 138.7 (C-16), 106.5 (C-17), 29.0 (C-18), 184.4 (C-19), 12.8 (C-20).

❖ **Compound TT5 (PDLN5 và PDLE4): 8(17), 13-ent-Labdadien-15→16-lactone-19-oic acid**

Yellowish oil, \([\alpha]_D = -44.6\) (MeOH)

- **PDLN5 (15 mg)**
  
  \(\text{\(^1H-NMR\) (CDCl\(_3\), 500 MHz):}\) \(\delta_H\) 7.10 (1H, \(s\), H-14), 4.89 và 4.59 (1H, \(s\) và 1H, \(s\), H-17), 4.67 (2H, \(d\), \(J = 1.5\) Hz, H-15), 1.24 (3H, \(brs\), H-18), 0.60 (3H, \(brs\), H-18). \(\text{\(^{13}C-NMR\) (CDCl\(_3\), 125 MHz):}\) \(\delta_C\) 39.2 (C-1), 19.9 (C-2), 37.9 (C-3), 44.2 (C-4), 56.3 (C-5), 26.0 (C-6), 38.6 (C-7), 147.4 (C-8), 55.7 (C-9), 40.5 (C-10), 21.9 (C-11), 24.7 (C-12), 134.9 (C-13), 143.9 (C-14), 70.1 (C-15), 174.4 (C-16), 106.8 (C-17), 29.0 (C-18), 183.2 (C-19), 12.8 (C-20).

- **PDLE4 (59 mg)**
  
  \(\text{\(^1H-NMR\) (CDCl\(_3\), 500 MHz):}\) \(\delta_H\) 7.11 (1H, \(d\), \(J = 1.0\) Hz, H-14), 4.89 và 4.59 (1H, \(s\) và 1H, \(s\), H-17), 4.77 (2H, \(d\), \(J = 1.5\) Hz, H-15), 1.24 (3H, \(brs\), H-18), 0.60 (3H, \(brs\), H-18). \(\text{\(^{13}C-NMR\) (CDCl\(_3\), 125 MHz):}\) \(\delta_C\) 39.2 (C-1), 19.9 (C-2), 37.9 (C-3), 44.2 (C-4), 56.3 (C-5), 26.0 (C-6), 38.6 (C-7), 147.4 (C-8), 55.7 (C-9), 40.5 (C-10), 21.9 (C-11), 24.7 (C-12), 134.9 (C-13), 143.9 (C-14), 70.1 (C-15), 174.4 (C-16), 106.8 (C-17), 29.0 (C-18), 183.8 (C-19), 12.8 (C-20).

❖ **Compound TT6 (PDWE9): Isopimaric acid (48)**

Colorless solid, 67 mg, ESI-MS: \(m/z\) 303.2 [M+H]+, 339.3 [M+Na]+, molecular formula: \(C_{20}H_{30}O_{2}\)
\(^{1}\text{H-NMR}\) (CDCl\(_3\), 500 MHz): \(\delta_H\ 5.80\ ((1H, dd, J = 17.5\ Hz, 11\ Hz, H-15),\ 5.32\ (1H, d, J = 4.0\ Hz, H-7),\ 4.92\ (1H, d, J = 17.0\ Hz, H-16a),\ 4.86\ (1H, d, J = 10.0\ Hz, H-16b),\ 2.03\ (1H, m, H-6e),\ 1.75\ (1H, m, H-6a),\ 0.91\ (3H, brs, H-20),\ 0.86\ (3H, brs, H-17),\ 0.27\ (3H, brs, H-19). \(^{13}\text{C-NMR}\) (CDCl\(_3\), 125 MHz): \(\delta_C\ 38.8\ (C-1),\ 17.9\ (C-2),\ 37.0\ (C-3),\ 44.3\ (C-4),\ 44.0\ (C-5),\ 25.2\ (C-6),\ 121.0\ (C-7),\ 135.7\ (C-8),\ 52.0\ (C-9),\ 35.0\ (C-10),\ 20.0\ (C-11),\ 36.1\ (C-12),\ 36.8\ (C-13),\ 46.1\ (C-14),\ 150.3\ (C-15),\ 109.3\ (C-16),\ 21.5\ (C-17),\ 185.4\ (C-18),\ 17.1\ (C-19),\ 15.3\ (C-20).

- **Compound TT7 (PNWE2): Totarol (321)**
  
  White powder, 33 mg, mp 128 °C

- **Compound TT8 (PNWE5) Totarol-19-carboxylic acid**
  
  White powder, 9 mg, ESI-MS (positive) \(m/z\ 353.10\ [M–2H+K]^+\), ESI-MS (negative) \(m/z\ 315.12\ [M–H]^–\)

- **Compound TT9 (PNWE4): Inumakiol D**
  
  Yellowish solid, 9 mg, mp 136 °C

- **Compound TT8 (PNWE5) Totarol-19-carboxylic acid**
  
  White powder, 9 mg, ESI-MS (positive) \(m/z\ 353.10\ [M–2H+K]^+\), ESI-MS (negative) \(m/z\ 315.12\ [M–H]^–\)

- **Compound TT9 (PNWE4): Inumakiol D**
  
  Yellowish solid, 9 mg, mp 136 °C
5), 31.2 (C-6), 65.5 (C-7), 134.2 (C-8), 140.4 (C-9), 38.9 (C-10), 124.2 (C-11), 117.1 (C-12), 154.4 (C-13), 133.4 (C-14), 28.1 (C-15), 20.6 (C-16), 20.7 (C-17), 28.6 (C-18), 181.1 (C-19), 22.5 (C-20).

❖ **Compound TT10**: PNWE1

Yellowish powder, 136 mg, IR (KBr) ν\textsubscript{max} cm\textsuperscript{-1}: 3547 (OH), 3095 (=C–H aromatic), 2954, 2875 (C–H aliphatic), 1700 (C=O/acid), 1374-1458 (C=C aromatic), 1249 (C=O/acid).

HR-ESI-MS (MeOH) m/z 653.3813 [M+Na]\textsuperscript{+} (calc. 653.3818)

\textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 500 MHz) and \textsuperscript{13}C-NMR (CD\textsubscript{3}Cl\textsubscript{3}, 125 MHz) (Table 3.12).

❖ **Compound TT11**: PKRE2

Yellowish powder, 12 mg, mp 119 °C

\textsuperscript{1}H-NMR (CD\textsubscript{3}OD, 500 MHz): δ\textsubscript{H} 8.07 (1H, d, J = 2.0 Hz, H-14), 7.73 (1H, dd, J = 8.5 Hz, 2.5 Hz, H-12), 7.44 (1H, brd, J = 8.5 Hz, H-11), 2.79 (1H, dd, J = 14.0 Hz, 2.0 Hz, H-5), 2.73 (1H, dd, J = 16.5 Hz, 14.0 Hz, H-6a), 2.52 (1H, dd, J = 17.0 Hz, 1.5 Hz, H-6b), 2.41 (1H, d, J = 12.0 Hz, H-1a), 1.87 (1H, m, H-2a), 1.75 (1H, m, H-2b), 1.66 (2H, m, H-3), 1.62 (1H, m, H-1b), 1.54 (6H, brs, H-16 và H-17), 1.31 (3H, brs, H-19), 1.28 (3H, brs, H-20). \textsuperscript{13}C-NMR (CD\textsubscript{3}OD, 125 MHz): δ\textsubscript{C} 38.7 (C-1), 19.9 (C-2), 38.5 (C-3), 46.0 (C-4), 45.8 (C-5), 39.2 (C-6), 202.1 (C-7), 131.7 (C-8), 156.4 (C-9), 38.8 (C-10), 124.9 (C-11), 132.0 (C-12), 148.9 (C-13), 124.0 (C-14), 72.6 (C-15), 31.7 (C-16/C17), 186.0 (C-18), 17.9 (C-19), 23.9 (C-20).

❖ **Compound TT12**: PDLE1

White powder, 10 mg, mp 247 °C

\textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 500 MHz): δ\textsubscript{H} 5.38 (1H, s, H-15), 3.19 (1H, dd, J = 11.5 Hz, 4.5 Hz, H-3a), 1.09 (3H, brs, H-30), 1.04 (3H, brs, H-29), 0.97 (3H, brs, H-23), 0.92 (3H, brs, H-28), 0.83 (3H, brs, H-26), 0.80 (3H, brs, H-25), 0.77 (3H, brs, H-24). \textsuperscript{13}C-NMR (CDCl\textsubscript{3}, 125 MHz): δ\textsubscript{C} 38.6 (C-1), 27.5 (C-2), 78.8 (C-3), 38.2 (C-4), 51.2 (C-5), 18.9 (C-6), 45.1 (C-7), 37.1 (C-8), 62.7 (C-9), 39.0 (C-10), 25.5 (C-11), 27.2 (C-12), 56.5 (C-13), 138.3 (C-14), 122.0 (C-15), 24.5 (C-16), 55.7 (C-17), 36.2 (C-18), 34.8 (C-19), 38.4 (C-20), 217.0 (C-21), 47.7 (C-22), 28.1 (C-23), 15.4 (C-24), 15.7 (C-25), 19.8 (C-26), 55.9 (C-27), 12.9 (C-28), 24.5 (C-29), 21.6 (C-30).
**Compound TF1 (PDWE1) Pinocembrin**

Colorless crystals, 700 mg, mp 192-193°C. ESI-MS: m/z 257.0 [M+H]+, molecular formula: C_{15}H_{12}O_{4}

^{1}H-NMR (CDCl₃, 500 MHz): δH 12.03 (1H, s, 5-OH), 7.39-7.47 (5H, m, H-2’→H-6’), 7.07 (1H, brs, 7-OH), 6.02 (1H, s, H-8), 6.01 (1H, s, H-6), 5.44 (1H, dd, J_{H2/H3a} = 13.0 Hz, J_{H2/H3e} = 3.5 Hz, H-2), 3.08 (1H, dd, J_{H3a/H3e} = 17.5 Hz, J_{H3a/H12} = 13.0 Hz, H-3a), 2.82 (1H, dd J_{H3e/H2} = 3.0 Hz, J_{H3e/H3a} = 17.5 Hz, H-3e). ^{13}C-NMR (CDCl₃, 125 MHz): δC 79.2 (C-2), 43.4 (C-3), 195.7 (C-4), 164.3 (C-5), 95.5 (C-6), 169.6 (C-7), 95.8 (C-8), 165.2 (C-9), 103.1 (C-10), 138.4 (C-1’), 126.2 (C-2’ và C-6’), 128.9 (C-3’ và C-5’), 128.9 (C-4’).

**Compound TF2 (PDWE2) Chrys

Yellow powder, 48 mg, mp 284-286 °C, ESI-MS: m/z 255.0 [M+H]+, molecular formula: C_{15}H_{10}O_{4}

^{1}H-NMR (CDCl₃ & CD₃OD, 500 MHz): δH 12.75 (1H, s, 5-OH), 7.90 (2H, dd, J = 7.9 Hz, 1 Hz, H-2’ và H-6’), 7.52-7.55 (3H, m, H-3’→H-5’), 6.66 (1H, s, H-3), 6.47 (1H, d, J = 2.0 Hz, H-6), 6.30 (1H, d, J = 2.0 Hz, H-8). ^{13}C-NMR (CDCl₃ & CD₃OD, 125 MHz): δC 164.3 (C-2), 105.0 (C-3), 182.7 (C-4), 161.8 (C-5), 99.6 (C-6), 164.3 (C-7), 94.6 (C-8), 158.3 (C-9), 105.5 (C-10), 131.4 (C-1’), 126.5 (C-2’ và C-6’), 129.2 (C-3’ và C-5’), 132.0 (C-4’).

**Compound TF3 (PDWE8) Pinostrobin**

Colorless solid, 137 mg, mp 112-113 °C

^{1}H-NMR (CDCl₃, 500 MHz): δH 12.01 (1H, s, 5-OH), 7.41-7.46 (5H, m, H-2’→H-6’), 6.06 (1H, d, J = 2.0 Hz, H-6), 6.05 (1H, d, J = 2.0 Hz, H-8), 5.40 (1H, dd, J_{H2/H3a} = 13Hz, J_{H2/H3e} = 3.0 Hz, H-2), 3.79 (3H, s, 7-OCH₃), 3.06 (1H, dd, J_{H3a/H3e} = 17.0 Hz, J_{H3a/H2} = 13.0 Hz, H-3a), 2.80 (1H, dd, J_{H3e/H2} = 17.0 Hz, J_{H3e/H3a} = 3.0 Hz, H-3e). ^{13}C-NMR (CDCl₃, 125 MHz): δC 79.2 (C-2), 43.3 (C-3), 195.7 (C-4), 162.7 (C-5), 95.1 (C-6), 167.9 (C-7), 94.2 (C-8), 164.1 (C-9), 103.1 (C-10), 138.4 (C-1’), 126.1 (C-2’ và C-6’), 126.1 (C-3’ và C-5’), 128.8 (C-4’), 55.6 (7-OCH₃).

**Compound TF4 (PKRE5 và PDWB2) (+) Catechin**, light yellow powder, [α]D = + 15.5 (MeOH)

- PKRE5 (120 mg)
1H-NMR (CD3OD, 500 MHz): $\delta_H$ 6.86 (1H, $d, J = 2$ Hz, H-2'), 6.79 (1H, $d, J = 8.0$ Hz, H-5'), 6.74 (1H, $dd, J = 8.0$ Hz, 2 Hz, H-6'), 5.95 (1H, $d, J = 2.5$ Hz, H-6), 5.88 (1H, $d, J = 2.5$ Hz, H-8), 4.59 (1H, $d, J = 7.5$ Hz, H-2), 3.99 (1H, $m, H$-3), 2.87 (1H, $dd, J = 16.0$ Hz, 5.5 Hz, H-4a), 2.53 (1H, $dd, J = 16.0$ Hz, 7.5 Hz, H-4b).

13C-NMR (CD3OD, 125 MHz): $\delta_C$ 82.9 (C-2), 68.8 (C-3), 28.5 (C-4), 157.6 (C-5), 96.3 (C-6), 157.8 (C-7), 95.5 (C-8), 156.9 (C-9), 100.9 (C-10), 132.2 (C-1'), 115.3 (C-2'), 146.2 (C-3'/C-4'), 116.1 (C-5'), 120.1 (C-6').

- PDWB2: 1H-NMR (CD3OD) (25 mg)

1H-NMR (CD3OD, 500 MHz): $\delta_H$ 6.86 (1H, $d, J = 1.5$ Hz, H-2'), 6.78 (1H, $d, J = 8.0$ Hz, H-5'), 6.74 (1H, $dd, J = 8.0$ Hz, 1.5 Hz, H-6'), 5.95 (1H, $d, J = 2$ Hz, H-6), 5.88 (1H, $d, J = 2.5$ Hz, H-8), 4.59 (1H, $d, J = 7.5$ Hz, H-2), 3.99 (1H, $m, H$-3), 2.87 (1H, $dd, J = 16.0$ Hz, 5.0 Hz, H-4a), 2.53 (1H, $dd, J = 16.0$ Hz, 8.5 Hz, H-4b).

- Compound TF5 (PDWE6) Kaempferol
  Yellow powder, mp 276-278 °C. ESI-MS: $m/z$ 287.1 [M+H]+

- Compound TF6 (PKRE8) 3'-O-Methylcatechin 7-O-β-D-glucopyranoside
  Yellow powder, 8 mg, [α]D = −144 (MeOH), ESI-MS: $m/z$ 467.2 [M+H]+, molecular formula: C22H26O11

1H-NMR (CD3OD, 500 MHz): $\delta_H$ 6.98 (1H, $d, J = 1.5$ Hz, H-2'), 6.86 (1H, $dd, J = 8.0$ Hz, 1.5 Hz, H-6'), 6.82 (1H, $d, J = 8.0$ Hz, H-5'), 6.30 (1H, $d, J = 2.5$ Hz, H-8), 6.05 (1H, $d, J = 2.5$ Hz, H-6), 4.84 (H-1", overlap với H2O), 4.64 (1H, $d, J = 8.0$ Hz, H-2), 4.05-4.01 (1H, $m, H$-3), 3.93 (1H, $d, J = 11.5$ Hz, H-6"a), 3.85 (3H, $s, 3'$-OCH3), 3.75 (1H, $dd, J = 11.5$ Hz, 4.5 Hz, H-6"b), 3.48-3.43 (m, H-2"- H-5"), 3.09 (1H, $dd, J = 16.5$ Hz, 5.5 Hz, H-4a), 2.60 (1H, $dd, J = 16.0$ Hz, 8.5 Hz, H-4b).

13C-NMR (CD3OD, 125 MHz): $\delta_C$ 83.1 (C-2), 68.7 (C-3), 28.8 (C-4), 158.0 (C-5), 98.1 (C-6), 156.7 (C-7), 97.0 (C-8), 158.1 (C-9), 103.9 (C-10), 123.2 (C-1''), 129.4 (C-2' và C-6''), 117.1 (C-3' và C-5''), 162.7 (C-4'').
Compound TF7 (PDLE7)  Kaempferol 3-O-(3′′′,6′′′-di-O-E-p-coumaroyl)-β-D-glucopyranoside

Yellow powder, 200 mg

\(^1H\)-NMR (CD\(_3\)OD, 500 MHz): \(\delta_H \) 7.98 (2H, \(d, J = 8.5 \text{ Hz}, \text{H}-2'/\text{H}6'\)), 7.69 (1H, \(d, J = 16.0 \text{ Hz}, \text{H}-7''\)), 7.45 (2H, \(d, J = 8.5 \text{ Hz}, \text{H}-2''/\text{H}6''\)), 7.41 (1H, \(d, J = 15.5 \text{ Hz}, \text{H}-7''\)), 7.27 (2H, \(d, J = 8.5 \text{ Hz}, \text{H}-2''/\text{H}6''\)), 6.82 (4H, \(d, J = 9.0 \text{ Hz}, \text{H}-3'/\text{H}5' \text{ và } \text{H}-3''''/\text{H}5''''\)), 6.80 (2H, \(d, J = 8.5 \text{ Hz}, \text{H}-3''/\text{H}5''\)), 6.42 (1H, \(d, J = 16.0 \text{ Hz}, \text{H}-8''\)), 6.26 (1H, brs, H-8), 6.13 (1H, brs, H-6), 5.07 (1H, \(d, J = 16.0 \text{ Hz}, \text{H}-8''\)), 5.39 (1H, \(d, J = 8.0 \text{ Hz}, \text{H}-1_g\)), 5.22 (1H, \(t, J = 9.5 \text{ Hz}, \text{H}-3_g\)), 4.38 (1H, \(d, J = 11.0 \text{ Hz}, \text{H}-6_g\)), 4.27 (1H, \(dd, J = 12.0 \text{ Hz}, \text{H}-7\)), 3.76 (1H, \(t, J = 9.5 \text{ Hz}, \text{H}-2_g\)), 3.72 (1H, \(dd, J = 7.0 \text{ Hz}, \text{H}-5_g\)), 3.63 (1H, \(m, \text{H}-4_g\)). \(^{13}C\)-NMR (CD\(_3\)OD, 125 MHz): \(\delta_C \) 159.1 (C-2), 135.2 (C-3), 179.1 (C-4), 162.7 (C-5), 100.0 (C-6), 165.7 (C-7), 94.9 (C-8), 158.1 (C-9), 105.5 (C-10), 122.5 (C-1'), 132.2 (C-2' và C6'), 116.0 (C-3' và C-5'), 161.4 (C-4'), 104.0 (C-1_g), 74.1 (C-2_g), 78.7 (C-3_g), 70.1 (C-4_g), 75.6 (C-5_g), 64.2 (C-6_g), 127.2 (C-1'''), 131.1 (C-2'' và C-6''), 116.8 (C-3'' và C-5''), 161.1 (C-4''), 146.8 (C-7''), 115.3 (C-8''), 169.0 (C-9''), 127.0 (C-1'''), 131.1 (C-2''' và C-6'''), 116.7 (C-3''' và C-5''''), 161.0 (C-4''''), 146.6 (C-7''''), 114.6 (C-8''''), 168.8 (C-9'''').

Compound TP1 (PDWE5) Dihydropinosylvin

Colorless oil, 110 mg, ESI-MS: \(m/z \) 215.1 [M+H] \(^+\), molecular formula: C\(_{14}\)H\(_{14}\)O\(_2\)

\(^1H\)-NMR (CDCl\(_3\), 500 MHz): \(\delta_H \) 7.21-7.25 (2H, \(m, \text{H}-3'/\text{H}-5'\)), 7.15-7.16 (1H, \(m, \text{H}-4'\)), 7.11-7.14 (2H, \(m, \text{H}-2'/\text{H}-6'\)), 6.22 (2H, \(d, J = 2.0 \text{ Hz}, \text{H}-2/\text{H}-6\)), 6.18 (1H, brs, H-4), 2.79-2.82 (2H-8, \(m\)), 2.71-2.74 (2H-7, \(m\)). \(^{13}C\)-NMR (CDCl\(_3\), 125 MHz): \(\delta_C \) 37.6 (C-7), 37.3 (C-8), 145.0 (C-1), 108.2 (C-2), 156.5 (C-3), 100.6 (C-4), 156.5 (C-5), 108.2 (C-6), 141.6 (C-1'), 128.4 (C-2' và C-6'), 128.3 (C-3' và C-5'), 125.9 (C-4').

Compound TP2 (PDWE11 và PDLN8) Dihydropinosylvin 5-methyl ether.

Colorless oil; mp 51 \(^{0}\)C
- PDWE11 (600 mg)

**1H-NMR** (CDCl$_3$, 500 MHz): $\delta_H$ 7.25-7.26 (2H, $m$, H-3'/H-5'), 7.14-7.18 (3H, $m$, H-4', H-2'/H-6'), 6.30 (1H, brs, H-4), 6.24 (2H, $t$, $J = 2.5$ Hz, H-2/H-6), 3.69 (3H, brs, O-CH$_3$) 2.83-2.87 (2H-8, $m$), 2.77-2.80 (2H-7, $m$). **13C-NMR** (CDCl$_3$, 125 MHz): $\delta_C$ 37.8 (C-7), 37.4 (C-8), 144.5 (C-1), 108.1 (C-2), 156.2 (C-3), 99.2 (C-4), 160.6 (C-5), 106.7 (C-6), 141.6 (C-1'), 128.4 (C-2' và C-6'), 128.3 (C-3' và C-5'), 125.9 (C-4'), 55.2 (5-OCH$_3$).

- PDLN8 (11 mg)

**1H-NMR** (CDCl$_3$, 500 MHz): $\delta_H$ 7.28-7.29 (2H, $m$, H-3'/H-5'), 7.17-7.20 (3H, $m$, H-4', H-2'/H-6'), 6.32 (1H, brs, H-4), 6.25 (2H, $m$, H-2/H-6), 3.74 (3H, brs, O-CH$_3$) 2.88-2.91 (2H-8, $m$), 2.81-2.85 (2H-7, $m$). **13C-NMR** (CDCl$_3$, 125 MHz): $\delta_C$ 37.9 (C-7), 37.6 (C-8), 144.5 (C-1), 108.0 (C-2), 156.6 (C-3), 99.1 (C-4), 160.9 (C-5), 106.8 (C-6), 141.7 (C-1'), 128.4 (C-2' và C-6'), 128.3 (C-3' và C-5'), 126.0 (C-4'), 55.3 (5-OCH$_3$).

- **Compound TP3 (PDWE12) 3-Hydroxy-5-methoxystilbene**

  Yellowish powder, mp 120 0°C

**1H-NMR** (CDCl$_3$, 500 MHz): $\delta_H$ 7.52 (2H, $d$, 7.0 Hz, H-2'/H-6'), 7.39 (2H, $t$, 7.5 Hz, H-3'/H-5'), 7.28-7.32 (1H, $m$, H-4'), 7.08 (1H, $d$, 16.0 Hz, H-7), 7.08 (1H, $d$, 16.5 Hz, H-8), 6.70 (1H, brs, H-6), 6.66 (1H, brs, H-2), 6.41 (1H, $t$, 2.0 Hz, H-4), 3.84 (3H, brs, O-CH$_3$). **13C-NMR** (CDCl$_3$, 125 MHz): $\delta_C$ 127.7 (C-7), 128.2 (C-8), 139.7 (C-1), 106.1 (C-2), 156.8 (C-3), 101.3 (C-4), 160.9 (C-5), 104.9 (C-6), 136.9 (C-1'), 126.6 (C-2' và C-6'), 128.6 (C-3' và C-5'), 129.4 (C-4'), 55.4 (5-OCH$_3$).

- **Compound TP4 (PKRE9) Resveratrol-3-O-β-D-glucoside**

- PKRE9

  Yellowish needles, 10 mg, mp 136 0°C, ESI-MS $m/z$ 391.1 [M+H]$^+$. molecular formula: C$_{20}$H$_{22}$O$_8$, Tên khác: (E) **piceid**

**1H-NMR** (CD$_3$OD, 500 MHz): $\delta_H$ 7.38 (2H, $d$, $J = 8.5$ Hz, H-2'/H-6'), 7.03 (1H, $d$, $J = 16.5$ Hz, H-8), 6.87 (1H, $d$, $J = 16.5$ Hz, H-7), 6.81 (1H, brs, H-2), 6.79 (2H, $d$, $J = 8.5$ Hz, H3'/H5'), 6.64 (1H, brs, H-6), 6.48 (1H, brs, H-4), 4.92 (1H, $d$, $J = 7.0$ Hz, H-1''), 3.95 (1H, $dd$, $J = 12.0$ Hz, 2.0 Hz, H-6''a), 3.74 (1H, $dd$, $J = 12.0$ Hz, 5.5 Hz, H-6''b), 3.40-3.52 (4H, $m$, H-2''-H-5''). **13C-NMR** (CD$_3$OD, 125 MHz): $\delta_C$ 141.4 (C-1), 107.1 (C-2), 160.4 (C-3), 104.1 (C-4), 10.
159.5 (C-5), 108.4 (C-6), 126.7 (C-7), 130.0 (C-8), 130.3 (C-1'), 129.0 (C-2' và C-6'), 116.5 (C-3' và C-5'), 158.4 (C-4'), glucose 102.4 (C-1''), 75.0 (C-2''), 78.1 (C-3''), 71.5 (-4'), 78.2 (C-5''), 62.6 (C-6'').

- **Mixture TP5** (PDWB1) the mixture of resveratrol-3-\(\beta\)-D-glucoside and 3,5,4'-trihydroxystilbene 4'-\(\beta\)-D-glucopyranoside

- **Resveratrol-3-\(\beta\)-D-glucoside**

\(^1\)H-NMR (CD\(_3\)OD, 500 MHz): \(\delta_H 7.38\) (2H, \(d, J = 8.5\) Hz, H-2'/H-6'), 7.04 (1H, \(d, J = 16.5\) Hz, H-8), 6.88 (1H, \(d, J = 16.5\) Hz, H-7), 6.81 (1H, brs, H-2), 6.79 (2H, \(d, J = 8.5\) Hz, H3'/H5'), 6.64 (1H, brs, H-6), 6.48 (1H, brs, H-4), 4.92 (1H, \(d, J = 7.0\) Hz, H-1''), 3.95 (1H, dd, \(J = 12.0\) Hz, 2.0 Hz, H-6''a), 3.74 (1H, dd, \(J = 12.0\) Hz, 5.5 Hz, H-6''b), 3.40-3.52 (4H, \(m, H-2''-H-5''\)). \(^{13}\)C-NMR (CD\(_3\)OD, 125 MHz): \(\delta_C 141.4\) (C-1), 107.0 (C-2), 160.5 (C-3), 104.1 (C-4), 159.6 (C-5), 108.4 (C-6), 126.7 (C-7), 130.0 (C-8), 130.3 (C-1'), 128.9 (C-2' và C-6'), 116.5 (C-3' và C-5'), 158.5 (C-4'), glucose 102.4 (C-1''), 78.2 (C-5''), 78.1 (C-3''), 75.0 (C-2''), 71.5 (-4''), 62.6 (C-6'').

- **Resveratroloside** (other name: 3,5,4'-trihydroxystilbene 4'-\(\beta\)-D-glucopyranoside)

\(^1\)H-NMR (CD\(_3\)OD, 500 MHz): \(\delta_H 7.47\) (2H, \(d, J = 8.5\) Hz, H-2'/H-6'), \(\delta_H 7.10\) (1H, \(J = 8.5\) Hz, H3'/H5'), 7.02 (1H, \(d, J = 16.5\) Hz, H-8), 6.90 (1H, \(d, J = 16.5\) Hz, H-7), 6.49 (2H, \(d, J = 2.0\) Hz, H-2 và H-6), 6.20 (1H, brs, H-4), 4.90 (1H, \(d, J = 7.0\) Hz, H-1''), 3.92 (1H, dd, \(J = 12.0\) Hz, 2.0 Hz, H-6''a), 3.74 (1H, dd, \(J = 12.0\) Hz, 5.5 Hz, H-6''b), 3.41-3.52 (4H, \(m, H-2''-H-5''\)). \(^{13}\)C-NMR (CD\(_3\)OD, 125 MHz): \(\delta_C 141.0\) (C-1), 106.0 (C-2 và C-6), 159.7 (C-3 và C-5), 104.1 (C-4), 128.6 (C-7), 129.9 (C-8), 133.2 (C-1'), 128.9 (C-2' và 6'), 117.9 (C-3' và 5'), 159.6 (C-4'), glucose 102.3 (C-1''), 78.2 (C-3''), 78.1 (C-5''), 75.0 (C-2''), 71.4 (C-4''), 62.5 (C-6'').

- **Compound TP6** (PDWB4) Vanillic acid 4-\((\beta\)-D-glucopyranoside)

Colourless powder, 13 mg, mp 137 °C

\(^1\)H-NMR (CD\(_3\)OD, 500 MHz): \(\delta_H 7.65\) (1H, s, H-2), 7.62 (1H, \(d, J = 8.5\) Hz, H-6), 7.19 (1H, \(d, J = 8.5\) Hz, H-5), 5.01 (1H, \(d, J = 7.0\) Hz, H-1''), 3.55 (1H, \(m, H-2''\)), 3.51 (1H, \(t, J = 9.0\) Hz, H-3''), 3.44 (1H, \(t, J = 8.5\) Hz, H-4''), 3.45 (1H, \(m, H-5''\)), 3.89 (1H, m, H-6'a), 3.72 (1H, \(m, H-6''b\)). \(^{13}\)C-NMR (CD\(_3\)OD, 125 MHz): 173.0 (C=O/ carboxylic acid), 130.0 (C-1), 114.6 (C-2), 150.0 (C-
3), 150.8 (C-4), 116.4 (C-5), 124.3 (C-6), glucose 102.2 (C-1'), 74.8 (C-2'), 77.8 (C-3'), 71.3 (C-4'), 78.2 (C-5'), 62.4 (C-6'), 56.6 (3-OCH₃).

❖ Compound TP7 (PKRE11) 3,4-Dimethoxyphenyl 2-O-(3-O-methyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside

White solid, 14 mg, [α]D − 90.0 (MeOH)

¹H-NMR (CD₃OD, 500 MHz): δH 6.88 (1H, d, J = 9.0 Hz, H-5), 6.78 (1H, J = 2.5 Hz, H-2), 6.67 (1H, dd, J = 9.0 Hz, 2.5 Hz, H-6), 5.34 (1H, brs, H-1''), 4.91 (1H, d, J = 7.5, H-1'), 4.18 (1H, m, H-2''), 4.15 (1H, dd, J = 9.5 Hz, 6.0 Hz, H-5''), 3.92 (1H, dd, J = 12.0 Hz, 6.0 Hz, H-6'a), 3.83 (3H, brs, 3-OCH₃), 3.78 (3H, brs, 4-OCH₃), 3.70 (1H, dd, J = 12.0 Hz, 6.0 Hz, H-6'b), 3.43 (3H, brs, 3''-OCH₃), 1.33 (3H, d, J = 6.0 Hz, H-6''). ¹³C-NMR (CD₃OD, 125 MHz): δC 145.9 (C-1), 103.6 (C-2), 153.8 (C-3), 151.3 (C-4), 108.5 (C-5), 114.2 (C-6), 101.6 (C-1'), 79.0 (C-2'), 79.2 (C-3'), 71.7 (C-4'), 78.1 (C-5'), 62.6 (C-6'), 102.3 (C-1''), 68.0 (C-2''), 82.0 (C-3''), 72.7 (C-4''), 69.9 (C-5''), 18.2 (C-6''), 57.2 (4-OCH₃ và 3''-OCH₃), 56.5 (3-OCH₃).

❖ Compound TL1 (PDWE3) (+) Lariciresinol

White powder, 482 mg, [α]D + 15.5 (MeOH), mp 168 °C, ESI-MS m/z, molecular formula: C₂₀H₂₄O₆

¹H-NMR (CDCl₃, 500 MHz): δH 6.85 (1H, d, J = 2.0 Hz, H-2'), 6.84 (1H, d, J = 8.0 Hz, H-5'), 6.83 (1H, d, J = 7.5 Hz, H-6), 6.78 (1H, dd, J = 8.0 Hz, 1.5 Hz, H-6'), 6.67 (1H, d, J = 8.0 Hz, H-5), 6.66 (1H, d, J = 1.5 Hz, H-2), 4.78 (1H, d, J = 6.5 Hz, H-7'), 4.04 (1H, dd, J = 8.5 Hz, 6.5 Hz, H-9a), 3.88 (1H, dd, J = 11.0 Hz, 3.5 Hz, H-9'a), 3.85 (3H, brs, 3-OCH₃), 3.84 (3H, brs, 3'-OCH₃), 3.75 (1H, m, H-9'b), 3.72 (1H, d, J = 6.5 Hz, H-9b), 2.89 (1H, dd, J = 14.0 Hz, 5.2 Hz, H-7a), 2.71 (1H, m, H-8), 2.52 (1H, dd, J = 13.5 Hz, 11.0 Hz, H-7b), 2.40 (1H, m, H-8'). ¹³C-NMR (CDCl₃, 125 MHz): δC 132.3 (C-1), 111.3 (C-2), 146.7 (C-3), 144.0 (C-4), 114.5 (C-5), 121.2 (C-6), 33.3 (C-7), 42.4 (C-8), 72.9 (C-9), 134.8 (C-1'), 108.4 (C-2'), 146.7 (C-3'), 145.1 (C-4'), 114.3 (C-5'), 118.8 (C-6'), 82.9 (C-7'), 52.6 (C-8'), 60.8 (C-9'), 56.0 (3-OCH₃), 55.9 (3'-OCH₃).

❖ Compound TL2 (PKRE4) Cedrusin

White powder, 15 mg, [α]D = + 4.8 (MeOH)
\textbf{1H-NMR (CD$_3$OD, 500 MHz):} $\delta$H 7.00 (1H, $d$, $J = 1.5$ Hz, H-2), 6.86 (1H, $dd$, $J = 8.0$ Hz, 1.5 Hz, H-6), 6.79 (1H, $d$, $J = 8.5$ Hz, H-5), 6.62 (1H, $s$, H-6$'$), 6.59 (1H, $s$, H-2$'$), 5.51 (1H, $d$, $J = 6.0$ Hz, H-7), 3.80 (3H, $s$, 3-OCH$_3$), 3.58 (2H, $t$, $J = 6.5$ Hz, H-9$'$), 3.47 (1H, $q$, $J = 6.0$ Hz, H-8), 2.58 (2H, $t$, $J = 7.5$ Hz, H-7$'$), 1.81 (2H, quintet, $J = 7.0$ Hz, H-8$'$).

\textbf{13C-NMR (CD$_3$OD, 125 MHz):} $\delta$C 135.1 (C-1), 110.6 (C-2), 149.0 (C-3), 147.3 (C-4), 116.1 (C-5), 119.7 (C-6), 88.7 (C-7), 55.7 (C-8), 65.1 (C-9), 136.7 (C-1$'$), 117.0 (C-2$'$), 141.8 (C-3$'$), 146.6 (C-4$'$), 129.8 (C-5$'$), 116.7 (C-6$'$), 32.7 (C-7$'$), 35.8 (C-8$'$), 62.3 (C-9$'$), 56.4 (3-OCH$_3$).

\textbf{Compound TL3 (PDWB6 và PKRE12) Cedrusin-4-O-\(\beta\)-D-glucopyranoside}

- PDWB6: White powder, 13 mg

\textbf{1H-NMR (CD$_3$OD, 500 MHz):} $\delta$H 7.16 (1H, $d$, $J = 8.5$ Hz, H-5), 7.09 (1H, $d$, $J = 1.5$ Hz, H-2), 6.98 (1H, $dd$, $J = 8.5$ Hz, 1.5 Hz, H-6), 6.61 (1H, $s$, H-6$'$), 6.60 (1H, $s$, H-2$'$), 5.57 (1H, $d$, $J = 5.5$ Hz, H-7), 3.85 (3H, $s$, 3-OCH$_3$), 3.57 (2H, $t$, $J = 6.5$ Hz, H-9$'$), 2.58 (2H, $t$, $J = 7.5$ Hz, H-7$'$), 1.81 (2H, quintet, $J = 6.5$ Hz, H-8$'$).

\textbf{13C-NMR (CD$_3$OD, 125 MHz):} $\delta$C 138.7 (C-1), 111.1 (C-2), 150.8 (C-3), 147.4 (C-4), 118.0 (C-5), 119.3 (C-6), 88.1 (C-7), 55.9 (C-8), 62.5 (C-9), 136.9 (C-1$'$), 117.1 (C-2$'$), 142.0 (C-3$'$), 146.4 (C-4$'$), 129.4 (C-5$'$), 116.6 (C-6$'$), 32.7 (C-7$'$), 35.7 (C-8$'$), 62.3 (C-9$'$), 56.7 (3-OCH$_3$); glucose 102.8 (C-1$_g$), 74.9 (C-2$_g$), 77.8 (C-3$_g$), 71.3(C-4$_g$), 78.1(C-5$_g$), 65.2 (C-6$_g$).

- PKRE12: White powder, 15 mg

\textbf{1H-NMR (CD$_3$OD, 500 MHz):} $\delta$H 7.16 (1H, $d$, $J = 8.5$ Hz, H-5), 7.08 (1H, $s$, H-2), 6.97 (1H, $dd$, $J = 8.0$ Hz, H-6), 6.61 (1H, $s$, H-6$'$), 6.60 (1H, $s$, H-2$'$), 5.57 (1H, $d$, $J = 5.5$ Hz, H-7), 3.83 (3H, $s$, 3-OCH$_3$), 3.57 (2H, $t$, $J = 6.5$ Hz, H-9$'$), 2.58 (2H, $t$, $J = 7.5$ Hz, H-7$'$), 1.81 (2H, quintet, $J = 6.5$ Hz, H-8$'$).

\textbf{13C-NMR (CD$_3$OD, 125 MHz):} $\delta$C 138.6 (C-1), 111.2 (C-2), 150.9 (C-3), 147.5 (C-4), 118.1 (C-5), 119.4 (C-6), 88.2 (C-7), 55.9 (C-8), 62.5 (C-9), 136.9 (C-1$'$), 117.1 (C-2$'$), 141.9 (C-3$'$), 146.4 (C-4$'$), 129.5 (C-5$'$), 116.7 (C-6$'$), 32.7 (C-7$'$), 35.7 (C-8$'$), 62.3 (C-9$'$), 56.7 (3-OCH$_3$); glucose 102.8 (C-1$_g$), 74.9 (C-2$_g$), 77.8 (C-3$_g$), 71.3(C-4$_g$), 78.1(C-5$_g$), 65.2 (C-6$_g$).

\textbf{Compound TS1 (PNWE3 và PDLN2) \(\beta\)-Sitosterol}

\textbf{1H-NMR (CDCl$_3$, 500 MHz):} $\delta$H 5.35 (1H, $d$, $J = 5.2$ Hz, H-6), 3.52 (1H, $m$, H-3), 2.27 (2H, $m$), 1.01 (3H, s, H-19), 0.90 (3H, $d$, $J = 6.6$ Hz, H-21), 0.84
(3H, \( t, J = 7.5 \text{ Hz}, H-29 \)), 0.82 (3H, \( d, J = 6.9 \text{ Hz}, H-27 \)), 0.78 (3H, \( d, J = 6.9 \text{ Hz}, H-26 \)), 0.68 (3H, \( s, H-18 \)). ¹³C-NMR (CDCl₃, 125 MHz): δc 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.4 (C-4), 140.8 (C-5), 121.7 (C-6), 31.7 (C-7), 31.9 (C-8), 50.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.3 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.1 (C-17), 11.9 (C-18), 19.8 (C-19), 36.16 (C-20), 18.8 (C-21), 34.0 (C-22), 26.1 (C-23), 45.9 (C-24), 29.2 (C-25), 19.1 (C-26), 19.4 (C-27), 23.1 (C-28), 12.0 (C-29).

- Compound TS2 (PDLN4 và PNWE9) Daucosterol

  - PNWE9

  ¹¹H-NMR (DMSO, 500 MHz): δH 5.33 (1H, brs, H-6), 4.24 (1H, \( d, J = 8 \text{ Hz}, H-1' \)), 3.67 (1H, \( d, J = 10.5 \text{ Hz}, H-6'a \)), 3.47 (1H, m, H-6'b), 3.11 (1H, m, H-3, overlap với DMSO), 0.99 (3H, brs, H-19), 0.92 (3H, \( d, J = 6.5 \text{ Hz}, H-21 \)), 0.86 (3H, brs, H-27), 0.84 (3H, brs, H-27), 0.67 (3H, brs, H-18), các -OH khác của glucose 4.57-4.60 (3H, m, 2′-OH, 3′-OH, 4′-OH), 4.20 (6′-OH, brs). ¹³C-NMR (DMSO, 125 MHz): δc 36.6 (C-1), 33.2 (C-2), 76.7 (C-3), 38.2 (C-4), 140.3 (C-5), 120.7 (C-6), 31.1 (C-7), 31.2 (C-8), 49.5 (C-9), 36.0 (C-10), 22.5 (C-11), 41.6 (C-12), 41.6 (C-13), 56.0 (C-14), 23.5 (C-15), 29.0 (C-16), 55.3 (C-17), 11.5 (C-18), 19.2 (C-19), 35.2 (C-20), 18.3 (C-21), 27.4 (C-22), 45.1 (C-23), 31.2 (C-24), 28.7 (C-25), 19.3 (C-26), 18.8 (C-27), 23.6 (C-28), 11.4 (C-29), glucose 100.7 (C-1′), 70.1 (C-2′), 76.9 (C-3′), 73.4 (C-4′), 76.4 (C-5′), 61.1 (C-6′).

  - PDLN4

  ¹¹H-NMR (DMSO, 500 MHz): δH 5.32 (1H, brs, H-6), 4.21 (1H, \( d, J = 7.5 \text{ Hz}, H-1' \)), 3.64 (1H, \( dd, J = 11.0 \text{ Hz}, 4.5 \text{ Hz}, H-6'a \)), 3.45 (1H, m, H-6'b), 3.11 (1H, m, H-3), 0.96 (3H, brs, H-19), 0.90 (3H, \( d, J = 6.5 \text{ Hz}, H-21 \)), 0.82 (3H, brs, H-27), 0.80 (3H, brs, H-27), 0.65 (3H, brs, H-18), các -OH khác của glucose 4.87 (3′-OH, brs), 4.85 (2′-OH, brs), 4.84 (4′-OH, brs), 4.40 (6′-OH, \( t, J = 5.5 \text{ Hz} \)).

Chapter 3. RESULTS AND DISCUSSION

3.1. Isolated compounds from Pinus dalatensis

The phytochemical investigation of Pinus dalatensis wood and leaves led to 24 compounds, including 01 sesquiterpenoid, 06 diterpenoids, 01 triterpenoids, 06 flavonoids, 06 phenol derivatives, 02 lignans and 02 sterols.
Compound TT1, TT3, TT5 and TP6 were isolated from the genus *Pinus* for the first time.
Compound TP6: Vanillic acid 4-(-β-D-glucopyranoside)

Colorless, mp 137 °C, molecular formula: C_{14}H_{18}O_{9}

Compound TP6 was isolated as colorless needles with a molecular formula
of C_{14}H_{8}O_{9} on the basis of its NMR data and ESI-MS pseudo-molecular ion at \( m/z \) 331.1 [M+H]^+. \(^1\)H- and \(^{13}\)C-NMR spectral data showed the signals belonged to an ABX system of benzene ring at \( \delta_H \) 7.65 (1H, s, H-2), 7.62 (1H, d, 8.5 Hz, H-6) and 7.19 (1H, d, 8.5 Hz, H-5), a methoxy group [\( \delta_H \) 3.92 (3H, s) and \( \delta_C \) 56.6], a carboxy group [\( \delta_C \) 173.0]. The identity of the \( \beta \)-D-glucopyranoside was confirmed by the comparison its NMR data with 11 (\( \delta_H \) 3.44 - 5.01, \( \delta_C \) 62.4 -102.2). The \( \beta \)-configuration was confirmed by the coupling constant of the anomeric proton (\( \delta_H \) 5.01, \( d, J = 7.0 \) Hz). The connection of the glucose at C-4 was deduced from the correlation of C-4 (\( \delta_C \) 150.8) with the anomeric proton H-1' (\( \delta_H \) 5.01) in HBMC. Another obvious HMBC correlation was observed between \( \delta_H \) 3.92 and \( \delta_C \) 150.0, indicating methoxylation at C-3. Furthermore, the NMR spectra of TP6 showed signal patterns similar to those of vanillic acid 4-\( \beta \)-D-glucopyranoside [156]. Therefore, the structure of TP6 was elucidated as vanillic acid 4-\( \beta \)-D-glucopyranoside, a vanillic acid glycosid is being reported for the first time from a species of Pinus.

![Figure 3.4. Key HMBC correlations for TP6](image)

**Figure 3.4. Key HMBC correlations for TP6**

### 3.2. Isolated compounds from Pinus kesiya

From the ethyl acetate extract of Pinus Kesiya roots, 07 compound were isolated, comprising 01 abietane diterpenoid, 02 flavonoids, phenol derivatives and 02 lignans. This is the first time compound TT11, TF6, TP4 and TP7 were isolated from the genus Pinus.
Compound TP4: Resveratrol-3-\(O\)-\(\beta\)-D-glucoside

Other name: (E) *Piceid*, yellowish needles, mp 136 "C
Compound TP4 was isolated as yellowish crystals. Its molecular formula, C_{20}H_{22}O_{8}, was suggested from combined analysis of the positive ESI MS at m/z 391.1 [M+H]^+ and $^1$H-, $^{13}$C-NMR and DEPT spectra. The sugar moiety was easily identified from its characteristic signals in the $^1$H and $^{13}$C NMR spectra as $\beta$-D-glucopyranose, thus giving C_{14}H_{12}O_{3} as aglycone. The $\beta$-configuration was confirmed by the coupling constant of the anomeric proton (δ_H 4.92, d, J=7.0 Hz, H-1′′). Its $^{13}$C NMR and HSQC spectra, except glucose moiety, observed 14 carbons including 3.C_q, 9.CH. The occurrence of two separate aromatic rings was established by the presence of signals for an AA′BB′ type system at δ_H 7.38 (2H, d, J = 8.5 Hz, H-2′/H-6′) and 6.79 (2H, d, J = 8.5 Hz, H3′/H5′) revealing a 1,4-disubstituted benzene ring. Besides, three protons at δ_H 6.81 (1H, brs, H-2), 6.64 (1H, brs, H-6) and 6.48 (1H, brs, H-4) also indicated a 1,3,5-trisubstituted benzene ring. Two trans olefinic protons appeared as an AB spin system with large coupling constant at 7.03 and 6.87 (each 1H, d, J = 16.5 Hz, H-8 and H-7, respectively). The position of each hydrogen to corresponding carbon in TP4 were assigned by cross-peaks in the HSQC spectrum. In addition, the NMR data of TP4 and (E)-piceid [165] are similar, hence they were suggested for the same compound. This compound has also been found in red wine [166] and in roots of Polygonum cuspidatum [167], but this is the first isolation from the genus Pinus.

3.3. Isolated compounds from Podocarpus nerifolius

The phytochemical study of Podocarpus nerifolius wood led to 06 compounds, containing 03 totarane diterpenoids, 01 bis-dierpenoid and 02 sterols (TS1 and TS2). Compound TT8, TT9 and TT10 were isolated from the genus Pinus for the first time.
The IR spectrum exhibited absorption bands and stretch frequencies at 3547 (broad), 3095, 2954, 2875, 1700, 1249 are ascribable to hydroxy, carboxylic acid (OH), aromatic (\(=\text{C} – \text{H}\)), aliphatic (C–H), carbonyl, carboxyl (C–O) functions, respectively. The HR-ESI-MS displayed a sodiated pseudomolecular ion peak [M+Na]\(^{+}\) at \(m/z\) 653.3813 \((\text{calc.} 653.3818)\) which in conjunction with the NMR data indicated a molecular formula of \(\text{C}_{40}\text{H}_{54}\text{O}_{6}\). The \(^{13}\text{C}\)-NMR and HSQC indicated 20 resonances for amonomeric unit \((4.\text{CH}_{3} + 5.\text{CH}_{2} + 3.\text{CH} + 8.\text{C})\) of the dimer. The \(^{1}\text{H}\) NMR indicated the appearance of the aromatic singlet at \(\delta_{\text{H}}\) 6.86 \((s,\ H-11/H-11')\). The presence of hydroxyl groups were
concluded by the singlet at $\delta_H 5.01$. The signals of isopropyl group appeared at $\delta_H 1.35$ ($d, J = 7.0$ Hz), 1.31 ($d, J = 7.0$ Hz) and 3.30 ($brs$). The presence of quaternary methyl groups were confirmed by the singlets at $\delta_H 1.34$ and 1.00.

The $^{13}$C-NMR spectrum of TT10 are absolutely similar to those of the totarol-19-carboxylic acid (TT8) (Table 3.13). The position of each hydrogen to corresponding carbon in TT10 were assigned by cross-peaks in the HSQC spectrum and the comparison of TT8 [170]. Moreover, the strong HMBC correlation observed between H-11,11′ and C-12,12′ (Figure 3.7) also confirmed that TT10 is the dimer of TT8. The configuration at C-4 and C-5 are determined by the NOESY correlation from H-5 ($\delta_H 1.48$, $brd$, $J = 12.0$ Hz) to H-18 ($\delta_H 1.34$, $brs$) (Figure 3.7). All the above spectroscopic data help to infer that TT10 is bis-totarol-19-carboxylic acid, which is named as macrophylllic acid.

**Table 3.13. NMR data of TT10 and totarol-19-carboxylic acid (TT8), $[CDCl_3, \delta$ (ppm), $J$ (Hz)]**

<table>
<thead>
<tr>
<th>Vị trí</th>
<th>TT10 (CDCl₃)</th>
<th>Totarol-19-carboxylic acid (TT8) (CDCl₃)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DEPT $\delta_C$ $\delta_H$ HMBC (H$\rightarrow$C) NOESY (H$\rightarrow$H) $\delta_C$ (ppm)</td>
<td></td>
</tr>
<tr>
<td>1, 1’</td>
<td>CH₂ 40.3 $e$: 2.09, $brd$, (13.0) $a$: 1.30 (overlap với H-16, H-17) C-2, C-10 40.1</td>
<td></td>
</tr>
<tr>
<td>2, 2’</td>
<td>CH₂ 19.9 $e$: 1.91-1.94, $m$ $a$: 1.56, $brd$, (13.0) C-1, C-3 20.1</td>
<td></td>
</tr>
<tr>
<td>3, 3’</td>
<td>CH₂ 36.9 $e$: 2.19, $brd$, (13.5) $a$: 1.07, $td$, (13.5, 4.0) C-2, C-4, C-19 37.2</td>
<td></td>
</tr>
<tr>
<td>4, 4’</td>
<td>C 43.6 - - 43.8</td>
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</tr>
<tr>
<td>5, 5’</td>
<td>CH 52.4 1.48, $brd$, (12.0) C-19, C-20, C-18, C-6, C-7, C-10, C-4 H-18 52.1</td>
<td></td>
</tr>
<tr>
<td>6, 6’</td>
<td>CH₂ 21.1 $e$: 2.26, $dd$, (13.5, 6.0) C-5, C-7, C-10, C-8, 21.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7, 7’</td>
<td>CH₂</td>
</tr>
<tr>
<td>----</td>
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<td>-----</td>
</tr>
<tr>
<td>8, 8’</td>
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<td>18, 18’</td>
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<tr>
<td>13-OH</td>
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<td>5.01, s</td>
</tr>
</tbody>
</table>

* Low signal intensity; e: equatorial; a: axial

### 3.4. Biological activities of some isolated compounds

**CONCLUSION AND RECOMMENDATIONS**

❖ **Conclusion**

Two *Pinus* species (*Pinus dalatensis*, *Pinus kesiya*) and a *Podocarpus* species (*Podocarpus neriifolius*) in Vietnam were researched on phytochemical and biological activities

1. Chemical constituents of *Pinus dalatensis* wood and leaves.
Compounds from plant materials were isolated by using column chromatography. Their structures were determined by combining spectral analysis and comparison with reported data. Twenty-four compounds were obtained including:

- 01 sesquiterpenoid: caryolane-1β,9β-diol (TT1);
- 06 diterpenoid: 16-hydroxy-8(17),13-labdadien-15,16-olid-19-oic acid (TT2a), 15-hydroxypinusolidic acid (TT2b), 15-methoxypinusolic (TT3), lambertianic acid (TT4), 8(17),13-ent-labdadien-15→16-lactone-19-oic acid (TT5), isopimaric acid (TT6); 01 triterpenoid: 3β-hydroxy-14-serraten-21-one (TT12);
- 06 flavonoid: pinocembrin (TF1), chrysin (TF2), pinostrobin (TF3), catechin (TF4), kaempferol (TF5), kaempferol 3-O-(3′,6′-di-O-E-p-coumaroyl)-β-D-glucopyranoside (TF7);
- 05 hop chat stilbenoid: dihydropinosylvin (TP1), dihydropinosylvin 5-methyl ether (TP2), 5-O-methylpinosylvin (TP3), resveratrol-3-O-β-D-glucoside (TP4), resveratroloside (TP5a);
- 01 simple phenol: vanillic acid 4-(-β-D-glucopyranoside) (TP6);
- 02 lignan: (+) lariciresinol (TL1), cedrusin-4-O-β-D-glucopyranoside (TL3);
- 02 sterol: β-sitosterol (TS1), daucosterol (TS2).

We found that terpenoids are predominant components of leaves, while flavonoids and stilbenoids are more prevalent in wood. Compound TT1, TT3, TT5 and TP6 were isolated from the genus Pinus for the first time.

2. Chemical constituents of Pinus kesiya roots.

There were seven isolated compounds:

- 01 abietane diterpenoid: 7-oxo-15-hydroxy-dehydroabietic acid (TT11);
- 02 flavonoids: catechin (TF4), 3′-O-methylcatechin 7-O-β-D-glucopyranoside (TF6);
- 01 stilbenoid: resveratrol-3-O-β-D-glucoside (TP4);
- 01 simple phenol: 3,4-dimethoxyphenyl 2-O-(3-O-methyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside (TP7);
- 02 lignans: cedrusin (TL2), cedrusin-4-O-β-D-glucopyranoside (TL3).

Compound TF6, TP4 và TP7 were isolated from the genus Pinus for the first time.
3. Chemical constituents of *Podocarpus neriifolius* wood.

There were six isolated compounds:

- 03 totarane diterpenoids: totarol (TT7), totarol-19-carboxylic acid (TT8), inumakiol D (TT9);
- 01 bis–diterpenoid: macrophylllic acid (TT10);
- 02 sterols: β-sitosterol (TS1), daucosterol (TS2).

This is the first time, TT8, TT9 and TT10 were isolated from this species.

4. Initial evaluation of biological activities of isolated compounds

The result of cytotoxic assay showed that TT7 and TT10 had expressed fairly strong activities on KB cells.

Stilbenoid TP2 and TP3 possess fair inhibition to SK-LU-1, MCF-7 and Hep-G2, while TT10 showed moderate inhibition to those cell lines.

The effects of the major compounds TT2, TT6, TF1, TF3, TP2, and TL1 on the growth of OCI-AML cells were investigated by evaluating cell counts, cell death, and the cell cycle. As a result, TT2, TT6, TT10, TF1 and TP2 decreased the number of OCI cells after 24 hour of culture with different potencies. TT6 and TF1 blocked of DNA synthesis (S phase), while TP2 significantly decreased the number of cells during mitosis (G2/M phase). Compound TT10 and mixture TT2 decreased the number of OCI cells after 24 hour of culture by blockage of DNA synthesis (S phase) and mitosis (G2/M phase).

In summary, the results have fully answered the aimed and questions posted in this thesis. Among 34 isolated compounds, there are 07 compounds were isolated for the first time from a species of genus *Pinus*; 06 compounds and 01 mixture showed cytotoxic activity. All isolated compounds belong to the classes of natural products which were published in *Pinus* and *Podocarpus*.

❖ Recommendations

1. Continue to study on the chemical composition in other parts of these species.

2. Further research on biological activity as well as the mechanism of substances action.
NEW CONTRIBUTIONS OF THIS THESIS

1. In Vietnam, this is the first research on chemical constituents of conifer species: *Pinus dalatensis*, *Pinus kesiya* and *Podocarpus neriifolius*. Thirty-four compounds were isolated and determined.

2. Seven compounds were isolated from the genus *Pinus* for the first time, including: caryolane-1β,9β-diol, 15-methoxypinusolic, 8(17),13-ent-labdadien-15→16-lactone-19-oic, vanillic acid 4-(-β-D-glucopyranoside), 3′-O-methylcatechin 7-O-β-D-glucopyranoside, resveratrol-3-O-β-D-glucoside and 3,4-dimethoxyphenyl 2-O-(3-O-methyl-α-L-rhamnopyranosyl)-β-D-glucopyranoside.

3. The *in vitro* biological activities of some major compounds was investigated. The results indicate that, totarol and macrophylllic acid inhibit the growth of KB cells; dihydropinosylvin 5-methyl ether, 5-O-methylpinosylvin and macrophylllic acid were active in the cytotoxicity test on SK-LU-1, MCF-7 and Hep-G2 cell lines; isopimaric acid, pinocembrin, dihydropinosylvin 5-methyl ether, macrophylllic acid and the mixture of 16-hydroxy-8(17),13-labdadien-15,16-olid-19-oic acid và 15-hydroxypinusolidic acid displayed anti-proliferative activity against OCI-AML cells, the result has statistically significant.
LIST OF WORKS HAS BEEN PUBLISHED


2. **Nguyen Hoang Sa**, Nguyen Thanh Tam, Dao Duc Thien, Tran Duc Quan, Tran Van Sung, Trinh Thi Thuy, Các hợp chất phenol từ rễ Thông ba lá (*Pinus kesiya*), Tạp chí Hóa học, 2016, 54(6A), 40-43.


4. **Nguyen Hoang Sa**, Nguyen Thanh Tam, Nguyen Thi Hoang Anh, Tran Duc Quan, Dao Duc Thien, Dinh Thi Phong, Tran Van Sung, Trinh Thi Thuy, Chemical constituents from the leaves of *Pinus dalatensis* Ferré, *Natural Product Research (SCIE)*, 2017, accepted on 10/07/2017 (http://dx.doi.org/10.1080/14786419.2017.1350672).
