STUDY ON CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF KNEMA PACHYCARPA) AND KNEMA SAXATILIS

Major: Organic chemistry

Code: 9.44.01.14

SUMMARY OF CHEMISTRY DOPRAL THESIS

Ha Noi - 2020
This thesis was completed at: Graduate university of Science and Technology - Vietnam Academy of Science and Technology

Advisor 1: Dr. Le Nguyen Thanh
Advisor 2: Prof. Dr. Nguyen Van Hung

Reviewer 1:
Reviewer 2:
Reviewer 3:

The dissertation will be defended before the Evaluation Council of the doctoral dissertation at the Academy, meeting at the Academy of Science and Technology - Vietnam Academy of Science and Technology at ... hours ..., date ... month... 2020.

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- The Library of Graduate University of Science and Technology, Vietnam Academy of Science and Technology.
INTRODUCTION

1. The urgency of the thesis

The genus *Knema* (Myristicaceae) is commonly found in tropical countries like Asia, Africa, and Australia. *Knema* plants have been used in the traditional medicine for treatment of pimples, sores, and skin diseases. Previous chemical studies of *Knema* species have reported the isolation of anacardic acids, cardanols, resorcinols, acetophenones, lignans, stilbene, and flavonoids. *Knema* plant exhibited antibacterial, anti-inflammatory, antioxidant, cytotoxic, and acetylcholinesterase inhibitory activities. However, there are few researches on the chemical components and biological activities of *Knema* species growing in Vietnam. In the active screening of plant species in Vietnam, search for biologically active substances, we found several species *Knema* express cytotoxic activity and inhibition of enzyme acetylcholinesterase: Ethyl acetate extract of *Knema pachycarpa* exhibits enzyme inhibitory activity acetylcholinesterase 100% at a concentration of 10 µg/ml and inhibits 44% of cell human epidemoid carcinoma (KB) at a concentration of 1 µg/ml. Ethyl acetate extract from leaves and stems branches and fruits of the *Knema saxatilis* have a 100% inhibitory effect growth of human adrenocortical carcinoma cell lines (SW13) in concentration 5 µg/ml.

From above reasons, thesis title was chosen to be “Study on chemical constituents and biological activities of *Knema pachycarpa* and *Knema saxatilis*.”

2. The aim of the thesis:

Study on chemical constituents of two *Knema* species including *Knema pachycarpa* and *Knema saxatilis* growing in Vietnam.
Evaluate cytotoxic and cetylcholinesterase enzyme inhibitory activities of isolates to find out bioactive compounds

3. The main contents of the thesis
- Isolation of compounds from *Knema pachycarpa* and *Knema saxatilis*.
- Determination of chemical structures of isolated compounds.
- Evaluation of cytotoxic and acetylcholinesterase enzyme inhibitory activities of isolated compounds.
CHAPTER 1: OVERVIEW

Overview of national and international researches related to my study of the chemical constituents and biological activities of Knema genus.

1.1. Introduction to Knema genus

1.1.1. Plant characteristics of Knema genus

The genus Knema (Myristicaceae) is commonly found in tropical countries like Asia, Africa, and Australia. It comprises approximately 60 species in Southeast Asia but the evergreen forests in Vietnam carries at least 14 species of this genus.

1.1.2. The review of Trichosanthes in traditional medicine

Knema plants have been used in the traditional medicine for treatment of pimples, sores, and skin diseases.

1.1.3. The review of Knema chemical constituents

In recent years, there have been many studies on chemical constituents and biological activities of Knema species. According to published papers in the literature, the chemical constituents of the Knema genus include main classes: anacardic acids, cardanols, resorcinols, acetophenones, lignans, stilbene, and flavonoids. Especially, phenylalkylphenol derivatives are quite common compounds in the species of Knema. The chemical constituents studies mainly focused on 12 species: K.attenuata, K. austrosiamensis, K. elegans, K. furfuraceae, K. glauca, K. globularia, K. glomerata, K. hookeriana, K. laurina, K. patentinervia, K. stellata subsp. cryptocaryoides, and K. tenuinervia.

1.1.4. The review of Knema biological activities
Studies showed that *Knema* and its active principles possessed a wide range of biological activities such as antibacterial, antinematodal, anti-inflammatory, cytotoxicity, and acetylcholinesterase inhibitory activities.

1.1.5. **Acetylcholinesterase inhibitory activities (AChE)**

Acetylcholinesterase is involved in the termination of impulse transmission by rapid hydrolysis of the neurotransmitter acetylcholine in numerous cholinergic pathways in the central and peripheral nervous systems. Through reversible inhibition of acetylcholinesterase, the neurotransmitter acetylcholine is retained for relatively longer periods of time, and therefore may have beneficial effects on the memory retention, at least in the short term.
CHAPTER 2: PLANT MATERIALS AND STUDYING METHODS

2.1. Plant materials

The fruits, stems and leaves of *Knema pachycarpa* were collected in A Luoi, Thua Thien Hue, Vietnam in May 2015. The stem and leaves of *Knema saxatilis* were collected in Huong Hoa, Quang Tri, Vietnam in May 2006. The scientific names of those *Knema* were identified by Dr. Nguyen Quoc Binh, Vietnam national museum of nature, Vietnam Academy of Science and Technology.

2.2. Isolation methods

Thin layer chromatography (TLC), Column chromatography (CC).

2.3. Structural elucidation methods

High resolution electrospary mass spectrum (HR-ESI-MS), Nuclear magnetic resonance spectroscopy (NMR), Optical rotation \([\alpha]\), Gas chromatography mass spectrometr (GC/MS).

2.4. Biological assays

Acetylcholinesterase enzyme assay and Cytotoxic assay
CHAPTER 3: EXPERIMENT AND RESULTS

3.1. Isolation of compounds from *Knema pachycarpa*

*Knema pachycarpa* (Dried fruits powder 0,38 kg)

- D: Dichormethane, M: Methanol
- E: Ethylacetate, H: n-Hexan
- A: Acetonitril, W: H₂O
- c.c: Column chromatography
- TLC: Thin layer chromatography

**Extraction in methanol**
(3 x 1 litters)

**Methanol extract** (190 g)

*Suspended in water and successively partitioned with n-hexane and ethyl acetate*

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**n-hexan extract**

MDH (150 g)

- Silica gel c.c H/E (0-100% E)

---

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH3 0,5 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC3 3 mg
  - MC4 12 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC5 15 mg
  - MC6 16 mg
  - MC7 2,6 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC1 4 mg
  - MC6 15 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC2 16 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC3 3 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC4 12 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC5 15 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC6 16 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC7 2,6 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

- MDH1 1,8 g
  - Sephadex LH-20 D/M (2/8)
  - TLC-RP-18 A/W (8/2)
  - MC8 24 mg

**EtOAc extract**

MDE (2,3 g)

- Silica gel c.c H/E (0-100% E)

---

**Water layer**

**Figure 3.1.** The isolation scheme of compounds from fruits *K. pachycarpa*
**Figure 3.2.** The isolation scheme of compounds from stems *K. pachycarpa*
**Knema pachycarpa (Dried leaves powder, 2.0 kg)**

D: Dichlomethane, M: Methanol  
E: Ethylacetate, H: n-Hexan  
A: Acetone  
c.c: Column chromatography

**Extraction in methanol**  
(3 x 10 liters)

**Methanol extract (400 g)**  
*Suspended in water and successively partitioned  
with n-hexane and ethyl acetate*

---

**n-hexan extract**  
**LMCH (225 g)**  
Silica gel c.c  
H/E (0-100%)

LMCH2  
34 g  
Silica gel  
H/E (8/2)

LMCH3  
63.5 g  
Silica gel  
H/E (8/2)

LMCH2.1  
2.8 g  
Silica gel  
H/E (85/15)

LMCH3.9  
1.5 g  
Silica gel  
H/E (7/3)

LMCH2.12.3  
0.51 g  
Silica gel  
H/D (7/3)

LMCH2.12  
31.3 mg  
MC12

LMCH3  
5.2 mg  
MC13

---

**EtOAc extract**  
**LE (110 g)**  
Silica gel c.c  
H/E (0-100%)

LE1  
2.4 g  
Silica gel  
H/E (7/3)

LE6  
1.4 g  
Sephadex  
LH-20 D/M (1/9)

LE8  
2.7 g  
Sephadex  
LH-20 D/M (1/9)

LE9  
2 g  
Sephadex  
LH-20 D/M (1/9)

LE1.1  
0.17 g  
Sephadex  
LH-20 D/M (1/9)

LE1.2  
0.4 g  
Sephadex  
LH-20 D/M (1/9)

LE1.3  
0.2 g  
Sephadex  
LH-20 D/M (2/8)

LE1.4  
0.3 g  
Sephadex  
LH-20 D/M (1/9)

LE1.5  
0.5 g  
Sephadex  
LH-20 D/M (1/9)

LE1.6  
0.2 g  
Sephadex  
LH-20 D/M (1/9)

LE1.7  
11 mg  
Sephadex  
LH-20 D/M (9/1)

LE1.8  
50 mg  
Sephadex  
LH-20 D/M (9/1)

LE1.9  
70 mg  
Sephadex  
LH-20 D/M (9/1)

LE1.10  
50 mg  
Sephadex  
LH-20 D/M (9/1)

LE1.11  
kết tình trong M.  
MC21  
23 mg

MC14  
10 mg

MC18  
5 mg

MC19  
3 mg

MC20  
9 mg

---

**Figure 3.3.** The isolation scheme of compounds from leaves *K. pachycarpa*
3.2. Isolation of compounds from *Knema saxatilis*

*Knema saxatilis* (Dried stems powder 1.12 kg)

- Extraction in methanol
  - (3 x 3 liters)
- **Methanol extract** (400 g)
  - Suspended in water and successively partitioned with n-hexane, ethyl acetate and n-butanol

**n-hexan extract** (5 g)  **EtOAc extract** (10 g)  **n-butanol extract** TCDM (53 g)  **Water layer**

- Silica gel c.c
  - D/M (0-100%)

- TCDM3
  - 0.8 g
    - Sephadex LH-20
      - D/M (2/8)
    - TCDM3.4
      - 40 mg
    - TLC-silica gel
      - D/M (9/1)
      - MC14: 2 mg
      - MC24: 2.5 mg

- TCDM6
  - Sephadex LH-20
    - D/M (2/8)
  - TCDM6.2
    - 110 mg
  - Silica gel
    - H/A (85/15)
  - TCDM6.2.5
    - 50 mg
  - TLC-silica gel
    - D/M (9/1)
    - MC25: 6.3 mg
    - MC26: 3 mg

- TCDM8
  - 1.5 g
    - Sephadex LH-20
      - D/M (2/8)
    - TCDM8.5
      - 50 mg
    - Silica gel
      - D/A (8/1)
      - MC19: 4 mg

Figure 3.4. The isolation scheme of compounds from stems *K. saxatilis*
**Knema saxatilis (Dried leaves powder 1,1 kg)**

D: Dichlomethane, M: Methanol
E: Ethylacetate, H: n-Hexan
A: Acetone
c.c: Column chromatography

**Extraction in methanol**
(3 x 5 liters)

**Methanol extract** (110 g)

Suspended in water and successively partitioned with n-hexane, ethyl acetate and n-butanol

**n-hexan extract** (53 g)

**EtOAc extract** (39 g)

Silica gel c.c
H/A (0-100%)

**Figure 3.5.** The isolation scheme of compounds from leaves *K. saxatilis*

### 3.3. Physical properties and spectroscopic data of the isolated compounds

This section provides physical properties and spectroscopic data of 29 compounds from *K. pachycarpa* and *K. saxatilis*
## CHAPTER 4. DISCUSSIONS

### Table 4.1. Chemical constituents of n-hexane extract of *K. pachycarpa* fruits (GC/MS, %TIC)

<table>
<thead>
<tr>
<th>RT</th>
<th>Compound</th>
<th>%TIC</th>
<th>RT</th>
<th>Compound</th>
<th>%TIC</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Fatty acids</td>
<td></td>
<td></td>
<td>Anacardic acid (C15:1)</td>
<td>7.7</td>
</tr>
<tr>
<td>28.23</td>
<td>Myristic acid</td>
<td>2.0</td>
<td>45.09</td>
<td>Anacardic acid (C15:1)</td>
<td>5.4</td>
</tr>
<tr>
<td>32.13</td>
<td>Palmitic acid</td>
<td>0.9</td>
<td>45.12</td>
<td>Anacardic acid (C15:0)</td>
<td>5.0</td>
</tr>
<tr>
<td>35.10</td>
<td>Stearic acid</td>
<td>0.5</td>
<td>47.47</td>
<td>Anacardic acid (C17:1)</td>
<td>1.5</td>
</tr>
<tr>
<td>35.22</td>
<td>Oleic acid</td>
<td>4.3</td>
<td>47.68</td>
<td>Anacardic acid (C17:1)</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>Cardanol (C13:0)</td>
<td></td>
<td>47.85</td>
<td>Anacardic acid (C17:1)</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>Cardanol (C15:1)</td>
<td>2.4</td>
<td>45.69</td>
<td>Acetophenones</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>(isomer)</td>
<td></td>
<td></td>
<td>Acetophenone (C15:1)</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>Cardanol (C15:1)</td>
<td>2.9</td>
<td>45.88</td>
<td>(isomer)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>(isomer)</td>
<td></td>
<td></td>
<td>Acetophenone (C17:1)</td>
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<td>1.6</td>
<td>48.31</td>
<td>(isomer)</td>
<td>0.8</td>
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<tr>
<td></td>
<td>Cardanol (C17:1)</td>
<td>0.6</td>
<td>48.46</td>
<td>Lignans</td>
<td>4.9</td>
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<td></td>
<td>(isomer)</td>
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<td>Seasamin</td>
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<td>48.98</td>
<td>Pluviatilol</td>
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<td></td>
<td>Piperitol</td>
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<td>Pinoresinol</td>
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<td></td>
<td>(isomer)</td>
<td></td>
<td></td>
<td>epi-Pinoresinol</td>
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</tr>
<tr>
<td></td>
<td>Cardanol (C19:1)</td>
<td>0.1</td>
<td>50.26</td>
<td>Unknown compounds</td>
<td>8.9</td>
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<td></td>
<td>Cardols</td>
<td>4.7</td>
<td>51.50</td>
<td>Hợp chất K1</td>
<td>0.1</td>
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<tr>
<td></td>
<td>Tr.</td>
<td></td>
<td></td>
<td>Hợp chất K2</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Cardol (C13:0)</td>
<td>0.8</td>
<td>57.53</td>
<td>Hợp chất K3</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>Cardol (C15:1)</td>
<td>1.6</td>
<td>53.33</td>
<td>Hợp chất K4</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>Cardol (C17:1)</td>
<td>2.1</td>
<td>45.32</td>
<td>Hợp chất K5</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>(isomer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anacardic acids</td>
<td>37.5</td>
<td>58.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anacardic acid (C13:1)</td>
<td>0.1</td>
<td>50.74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**TIC**: Total Ion Chromatogram  
**RT**: Retention time
4.1. Determination of chemical structures of isolated compounds

4.1.1. Compound MC1: Acid knepachycarpic A (new compound)

Compound MC1 was isolated as a white amorphous powder. The molecular formula, C_{26}H_{34}O_{5}, was determined from the quasi-molecular ion peak at m/z: 425.2350 [M–H]^{-} in the negative HR-ESI-MS of MC1. The IR spectrum showed hydroxyl and carbonyl absorptions at 3427 and 1646 cm^{-1}, respectively. The ¹H-NMR spectrum of MC1 exhibited signals of an anacardic acid derivative with ABC-type protons including two doublets at δ_{H} 6.84 (1H, d, J = 8.0 Hz, H-3) and 6.75 (1H, d, J = 8.0 Hz, H-5), and a triplet at δ_{H} 7.33 (1H, t, J = 8.0 Hz, H-4). Additionally, signals of a 3,4-methylenedioxyphenyl moiety were observed: doublets at δ_{H} 6.66 (1H, d, J = 1.0 Hz, H-2"), 6.71 (1H, d, J = 8.0 Hz, H-5") and 6.61 (1H, br d, J = 8.0 Hz, H-6"), along with a singlet at δ_{H} 5.91 (2H, s). Correlations in HMBC spectrum were found between the methylene protons (δ_{H} 5.91) and oxygenated quaternary carbons at δ_{C} 147.4 (C–3") and 145.3 (C–4"). From NMR (¹H, ¹³C and HSQC) and HR-MS data, presence of a saturated aliphatic chain was also deduced besides signals of a carboxylic carbon at δ_{C} 174.1, 12 aromatic carbons and a methylenedioxy carbon at δ_{C} 100.6. All presented data of compound MC1 were very similar to those of kneglobularic acid B. The main difference was the numbers of methylene carbons linked between the two phenyl groups. By using the information of
HR-MS, the data supported that the linker consists of twelve methylenegroups. This was then confirmed by the HMBC correlations of H-1′ ($\delta_H$ 2.95) with C-1 ($\delta_C$ 110.5), C-5 ($\delta_C$ 122.5), C-6 ($\delta_C$ 147.4) and C-2′ ($\delta_C$ 32.0); correlations of proton H-12′ ($\delta_H$ 2.50) with C-1″ ($\delta_C$ 136.8), C-2″ ($\delta_C$ 108.8) and C-11′ ($\delta_C$ 31.7) these data, the structure of MC1 was determined as 2-hydroxy-6-(12′-(3″,4″-methylendioxyphenyl)dodecyl)-benzoic acid, named as knepachycarpic acid A.

The structure of knepachycarpic acid A is confirmed by GC/MS data after trimethylsilylation. The GC/MS analysis showed compound MC1 reported the same Rf of compound K1. Compound MC1-diTMS weak molecular ion peaks were observed at $m/z$ 570 (C$_{32}$H$_{50}$O$_5$Si$_2$) respectively. The first fragment was obtained from the loss of a methyl group with $m/z$ 555 ([(M+2TMS)-15 (CH$_3$)]$^+$). The [(M+2TMS)-90(OTMS)]$^+$ 480 $m/z$ described the loss of a OTMS group. The fragment with $\gamma$-cleavage of the side chain gave rise to the common fragment ion $m/z$ 219 (C$_{12}$H$_{15}$O$_2$Si). Fragment ion at $m/z$ 219 has been regarded as characteristic for aromatic moiety of anacardic acid derivatives, while the peak at $m/z$ 135 (C$_8$H$_7$O$_2$) was attributed to the presence of amethylenedioxybenzyl moiety.

**Hình 4.2.** Fragmentation pattern of MC1 di-trimethylsilyl
<table>
<thead>
<tr>
<th>C</th>
<th>Kneglobularic acid B</th>
<th></th>
<th>MC1</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>110.6</td>
<td>-</td>
<td>110.5</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>163.5</td>
<td>-</td>
<td>163.5</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>115.8</td>
<td>6.87, d (8.4)</td>
<td>115.7</td>
<td>6.84, d (8.0)</td>
</tr>
<tr>
<td>4</td>
<td>135.3</td>
<td>7.36, t (8.4)</td>
<td>135.0</td>
<td>7.33, t (8.0)</td>
</tr>
<tr>
<td>5</td>
<td>122.7</td>
<td>6.77, d (8.4)</td>
<td>122.5</td>
<td>6.75, d (8.0)</td>
</tr>
<tr>
<td>6</td>
<td>147.7</td>
<td>-</td>
<td>147.4</td>
<td>-</td>
</tr>
<tr>
<td>1'</td>
<td>36.4</td>
<td>2.98, t (8.0)</td>
<td>36.4</td>
<td>2.95, t (7.5)</td>
</tr>
<tr>
<td>2'</td>
<td>31.9</td>
<td>1.50-1.65, m</td>
<td>32.0</td>
<td>1.53-1.60, m</td>
</tr>
<tr>
<td>3'</td>
<td>29.7</td>
<td>1.20-1.42, m</td>
<td>29.8</td>
<td>1.29-1.25, m</td>
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<tr>
<td>4'</td>
<td>29.1</td>
<td>1.20-1.42, m</td>
<td>29.6</td>
<td>1.29-1.25, m</td>
</tr>
<tr>
<td>5'</td>
<td>29.4</td>
<td>1.20-1.42, m</td>
<td>29.6</td>
<td>1.29-1.25, m</td>
</tr>
<tr>
<td>6'</td>
<td>29.4</td>
<td>1.20-1.42, m</td>
<td>29.59</td>
<td>1.29-1.25, m</td>
</tr>
<tr>
<td>7'</td>
<td>31.7</td>
<td>1.53-1.60, m</td>
<td>29.55</td>
<td>1.29-1.25, m</td>
</tr>
<tr>
<td>8'</td>
<td>35.6</td>
<td>2.50, t (7.5)</td>
<td>29.47</td>
<td>1.29-1.25, m</td>
</tr>
<tr>
<td>9'</td>
<td>-</td>
<td>-</td>
<td>29.47</td>
<td>1.29-1.25, m</td>
</tr>
<tr>
<td>10'</td>
<td>-</td>
<td>-</td>
<td>29.1</td>
<td>1.29-1.25, m</td>
</tr>
<tr>
<td>11'</td>
<td>-</td>
<td>-</td>
<td>31.7</td>
<td>1.53-1.60, m</td>
</tr>
<tr>
<td>12'</td>
<td>-</td>
<td>-</td>
<td>35.6</td>
<td>2.50, t (7.5)</td>
</tr>
<tr>
<td>1''</td>
<td>136.8</td>
<td>-</td>
<td>136.8</td>
<td>-</td>
</tr>
<tr>
<td>2''</td>
<td>108.8</td>
<td>6.67, s</td>
<td>108.8</td>
<td>6.66, d (1.0)</td>
</tr>
<tr>
<td>3''</td>
<td>147.4</td>
<td>-</td>
<td>147.4</td>
<td>-</td>
</tr>
<tr>
<td>4''</td>
<td>145.3</td>
<td>-</td>
<td>145.3</td>
<td>-</td>
</tr>
<tr>
<td>5''</td>
<td>108.0</td>
<td>6.71, d (8.0)</td>
<td>108.0</td>
<td>6.71, d (8.0)</td>
</tr>
<tr>
<td>6''</td>
<td>121.0</td>
<td>6.61, d (8.0)</td>
<td>121.0</td>
<td>6.61, brd (8.0)</td>
</tr>
<tr>
<td>OCH$_2$O</td>
<td>100.6</td>
<td>5.91, s</td>
<td>100.6</td>
<td>5.90, s</td>
</tr>
<tr>
<td>COOH</td>
<td>175.9</td>
<td>-</td>
<td>174.1</td>
<td>-</td>
</tr>
</tbody>
</table>

a: 500MHz. CDCl$_3$; b: 125MHz. CDCl$_3$; c: 400MHz. CDCl$_3$; d: 100MHz. CDCl$_3$
4.1.2. Chemical structure of isolated compounds

This section presents the detailed results of spectral analysis and structure determination of 33 isolated compounds from *K. pachycarpa* and *K. saxatilis.*

* 23 compounds from *K. pachycarpa* (Figure 4.4), including: 8 new compounds (MC1, MC2, MC3, MC4, MC5, MC9, MC10, MC11), 11 compounds (MC6, MC7, MC8, MC12, MC13, MC15, MC17, MC18, MC21, MC22, MC23) were reported from *Knema* genus for the first time and 9 known.
Figure 4.4. Chemical structure of compounds from *K. pachycarpa*
* 10 compounds from *K. saxatilis* (Figure 4.5), including: 3 compounds (MC24, MC28, MC29) were reported from *Knema* genus for the first time and 7 known, 4 compounds isolated from *K. pachycarpa* and *K. saxatilis* (MC14, MC16, MC19, MC20).

**Figure 4.5.** Chemical structure of compounds from *K. saxatilis*
4.2. Biological activities results
4.2.1. Acetylcholinesterase inhibitory activity of compounds from *K. pachycarpa*

*Table 4.3. Acetylcholinesterase inhibitory activity of compounds from *K. pachycarpa***

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knepachycarpic acid A (MC1)</td>
<td>8,19</td>
</tr>
<tr>
<td>Knepachycarpic acid B (MC2)</td>
<td>3,89</td>
</tr>
<tr>
<td>Knepachycarpanol A (MC3)</td>
<td>2,60</td>
</tr>
<tr>
<td>Knepachycarpanol B (MC4)</td>
<td>7,09</td>
</tr>
<tr>
<td>Knepachycarpasinol (MC5)</td>
<td>2,46</td>
</tr>
<tr>
<td>Knepachycarpanone A (MC9)</td>
<td>1,74</td>
</tr>
<tr>
<td>Knepachycarpanone B (MC10)</td>
<td>0,72</td>
</tr>
<tr>
<td>Knepachycarpanol C (MC11)</td>
<td>3,35</td>
</tr>
<tr>
<td>Globulol (MC12)</td>
<td>23,06</td>
</tr>
<tr>
<td>Biochanin A (MC14)</td>
<td>73,07</td>
</tr>
<tr>
<td>5,7,3'-Trihydroxy-5'-methoxyl-isoflavone (MC15)</td>
<td>NA</td>
</tr>
<tr>
<td>Luteolin (MC16)</td>
<td>NA</td>
</tr>
<tr>
<td>Chrysoeriol (MC17)</td>
<td>NA</td>
</tr>
<tr>
<td>(+) - Catechin (MC19)</td>
<td>NA</td>
</tr>
<tr>
<td>Hydnocarpin D (MC22)</td>
<td>NA</td>
</tr>
<tr>
<td>Donepezil</td>
<td>0,12</td>
</tr>
</tbody>
</table>

NA: Not active
4.2.2. Cytotoxic activity of compounds from *K. pachycarpa*

*Table 4.4. Cytotoxic activity of new compounds*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (µM)</th>
<th>Hela</th>
<th>MCF-7</th>
<th>Hep3B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kneepachycarpic acid A (MC1)</td>
<td>91,20</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Kneepachycarpic acid B (MC2)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Kneepachycarpanol A (MC3)</td>
<td>81,28</td>
<td>82,74</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Kneepachycarpanol B (MC4)</td>
<td>33,11</td>
<td>31,36</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Kneepachycarpasinol (MC5)</td>
<td>32,36</td>
<td>41,30</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Kneepachycarpanone A (MC9)</td>
<td>26,92</td>
<td>52,88</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Kneepachycarpanone B (MC10)</td>
<td>30,20</td>
<td>46,22</td>
<td>70,80</td>
<td></td>
</tr>
<tr>
<td>Kneepachycarpanol C (MC11)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Camptothecin</td>
<td>0,15</td>
<td>0,20</td>
<td>0,20</td>
<td></td>
</tr>
</tbody>
</table>

NA: Not active

4.2.2. Cytotoxic activity of compounds from *K. saxatilis*

*Table 4.5. Cytotoxic activity of compounds from K. saxatilis*

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (µM)</th>
<th>Lu</th>
<th>MCF-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eriodictyol (MC24)</td>
<td>159,7</td>
<td>218,4</td>
<td></td>
</tr>
<tr>
<td>Sulfuretin A (MC25)</td>
<td>237,0</td>
<td>355,5</td>
<td></td>
</tr>
<tr>
<td>Taxifolin (MC26)</td>
<td>65,8</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Ellipticin</td>
<td>1,5</td>
<td>1,9</td>
<td></td>
</tr>
</tbody>
</table>

NA: Not active
CONCLUSIONS

1. From the fruits, stems, leaves of *K. pachycarpa* growing in Vietnam, we isolated and determined 23 compounds, including:
   ✓ **8 new compound**: knepachycarpic acid A (MC1), knepachycarpic acid B (MC2), knepachycarpanol A (MC3), knepachycarpanol B (MC4), knepachycarpasanol (MC5), knepachycarpanone A (MC9), knepachycarpanone B (MC10), knepachycarpanol C (MC11).
   ✓ 11 compounds were isolated from *Knema* genus for the first time:
      ✓ 3 compounds lignan: (+)-Pinoresinol (MC6), (+)-epipinoresinol (MC7), Piperitol (MC8).
      ✓ 6 compounds flavonoid: 5,7,3'-Trihydroxy-5'-methoxyl-isoflavone (MC15), Chrysoeriol (MC17), Naringenin (MC18), Kaempferol-3-O-rutinoside (MC21), hydnocarpin D (MC22), Hydnocarpin (MC23).
      ✓ 2 compounds terpene: Globulol (MC12), Caryolol (MC13).

2. From the stems, leaves of *K. saxatilis* growing in Vietnam, we isolated and determined 10 compounds, including:
   ✓ 3 compounds were isolated from *Knema* genus for the first time:
      ✓ 1 compound flavonoid: Eriodictyol (MC24)
      ✓ 1 compound sesquiterpene: Clovan-2β,9α-diol (MC28)
      ✓ 1 compound lignan: (+)-Isolariciresinol (MC29)

3. The acetylcholinesterase (AChE) inhibitory activity of n-hexane extract of *K. pachycarpa* fruits and 15 compounds isolated from *K. pachycarpa*. The n-hexane extract showed very strong AChE inhibitory activity with IC$_{50}$ of 0.05 μg/ml. Strong AChE inhibition with IC$_{50}$ values ranging from 0.72 μM to 8.19 μM were also found for all 15 isolated compounds.
Among them, compound **MC10** were the most active with **IC**\textsubscript{50} values of 0.72 μM, respectively.

4. The cytotoxic evaluation of the new compounds against three cancer cell lines including Hep3B (human hepatoma cancer), HeLa (human cervical adenocarcinoma) and MCF-7 (human breast adenocarcinoma). Among them, compound **MC10** showed cytotoxic against three cells with the **IC**\textsubscript{50} values of 70,80 μM, 46,22 μM, 30,20 μM, respectively.

**RECOMMENDATIONS**

From the results of chemical constituents and biological activities of *K. pachycarpa, K. saxatilis*, we demonstrate that:

Compound **MC10** from *K. pachycarpa* exhibited strong inhibitory acetylcholinesterase activity and cytotoxic activity on three human cancer cell lines Hep3B, MCF-7, Hela. Therefore, further studies (in vivo model) of this compound are required.
NEW FINDINGS OF THE THESIS

1. This is the first study on the chemical constituents and biological activities of *K. pachycarpa* and *K. saxatilis* grown Vietnam.

2. 8 new compounds and 14 compounds were isolated for the first time from *Knema* genus. The new compounds were named as knepachycarpic acid A (MC1), knepachycarpic acid B (MC2), knepachycarpanol A (MC3), knepachycarpanol B (MC4), knepachycarpasinol (MC5), knepachycarpanone A (MC9), knepachycarpanone B (MC10), knepachycarpanol C (MC11).

3. 8 new compounds and several compounds isolated from *K. pachycarpa* and *K. saxatilis* have been evaluated for acetylcholinesterase inhibitory activity and cytotoxic activity for the first time.
PUBLICATIONS WITHIN THE SCOPE OF THESIS


