Vietnam Journal of Science and Technology ***61*** *(2) (2023) 182-189*

doi:10.15625/2525-2518/17571

Phenolic compounds from *knema pachycarpa* leaves

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Received: 10 October 2022; Accepted for publication: 30 January 2023

Abstract. *Knema* (Myristicaceae family) is a large genus, comprising over 100 species found in of South East Asian countries, among that over 10 species was found in Vietnam. *Knema pachycarpa* is a native tree distributed in central provinces of Viet Nam. Until now, only few chemical studies of *Knema* species in Viet Nam have been reported. Phytochemical study of *Knema pachycarpa* leaves led to the isolation of seven known compounds including six flavonoids 5,7,3′-trihydroxy-5′-methoxy isoflavone (**1**), luteolin (**2**), (-)-narigenin (**3**), (+)-catechin (**4**), isoquercetrin (**5**), and nicotiflorin (**6**), and a biphenyl compound 3,3',4,4'-tetrahydroxybiphenyl (**7**). Their structures were elucidated by MS and NMR data analysis and compared with those previously reported. Compounds **3** and **6**-**7** were found from *Knema* genus for the first time. In the cytotoxic assay, biphenyl compound (**7**) exhibited strong cytotoxicity to KB cancer cell line with IC_{50} value of 2.38 ± 0.11 μg/mL. Compound **7** also displayed showed activity against HepG2, Lu and MCF7 cancer cell lines with IC_{50} ranges from 18.25 ± 0.92 μg/mL to 35.56 ± 1.17 μg/mL, whereas flavonoids **1**-**6** were weakly active or inactive against tested cell lines.

Keywords: Knema pachycarpa, biphenyl, flavonoids.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Knema (Myristicaceae family) is a large genus, comprising over 100 species found in countries of South East Asian region [1]. *Knema* species have been used in the folk medicine to treat skin diseases, rheumatism and cancers [2]. Previous chemical investigations revealed the presence of phenol lipid derivatives, lignans, stilbenes and flavonoids from *Knema* plants [2-4]. *Knema* plant extracts and its active compounds showed many pharmacological effects such as anticancer, antidiabetic, antiinflammatory and acetylcholinesterase inhibitory activities [2-4].

Plant *Knema pachycarpa* de Wilde, that is called "Mau cho trai day", is a native tree in Viet Nam and only few chemical studies of this *Knema* species have been reported by our group

[5-7]. In our biological activity screening of Vietnamese plants, the MeOH extract of leaves of *K. pachycarpa* showed cytotoxic activity against KB cell line with IC_{50} of 78.21 ± 4.35 μg/mL. In the continuation of our study on *Knema* plants, herein the chemical constituents of *K. pachycarpa* leaves were described. Seven phenolic compounds were isolated and elucidated as 5,7,3′-trihydroxy-5′-methoxy isoflavone (**1**), luteolin (**2**),(-)-narigenin (**3**), (+)-catechin (**4**), isoquercetrin (**5**), nicotiflorine (**6**), and 3,3',4,4'-tetrahydroxybiphenyl (**7**) by NMR and MS spectral data. The isolated compounds were evaluated for cytotoxicity against several cancer cell lines.

2. MATERIALS AND METHODS

2.1. Plant materials

The plant materials were collected at A-Luoi district, Hue province, Viet Nam, in May 2015 and identified as *Knema pachycarpa* de Wilde by Dr. Nguyen The Cuong, Melinh station for biodiversity. A voucher specimen (VN-1527) was stored at the Institute of Marine Biochemistry, Vietnam Academy of Science and Technology**.**

2.2. General experimental procedures

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded by a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh) or Sephadex® LH-20. Thin layer chromatography used precoated silica gel plates (Merck 60 F_{254}). Compounds were visualized by spraying with 10% sulfuric acid and heating.

2.3. Extraction and isolation

The dried, powdered leaves of *K. pachycarpa* (2 kg) was extracted with MeOH at room temperature (4 times × 10 L for one day). The extracts were filtered and solvent was removed under reduced pressure. The MeOH residue was suspended in water (1 L) and the suspension was consecutively extracted with *n*-hexane and EtOAc. Evaporation of organic solvents under reduced pressure afforded *n*-hexane residue (225 g) and EtOAc residue (110 g), respectively. The EtOAc residue was subjected to silica gel CC and eluted with a gradient solvent system of *n*-hexane/EtOAc (100:1 – 0:1) to afford 15 fractions E1–E15, respectively. Fraction E3 (2.4 g) was separated by silica gel CC and eluted with *n*-hexane/EtOAc (8:2, v/v) to give 6 fractions E3.1-E3.6. Fraction E3.2 (0.4 g) was purified by Sephadex® LH-20 CC using MeOH/CH_2Cl_2 (8:2, v/v) as eluent to yield compound **1** (3 mg). Fraction E3.4 (0.3 g) was purified by Sephadex® LH-20 CC, eluted with MeOH/CH_2Cl_2 (9:1, v/v) to afford 2 sub-fractions E3.4.1 – E3.4.2. Fraction E3.4.2 (50 mg) was separated by preparative TLC using solvent mixture of CH_2Cl_2/acetone (9:1, v/v) to give compound **2** (5 mg) and **3** (3.5 mg). Fraction E7 (2.5 g) was separated with Sephadex® LH-20 CC and eluted with MeOH/CH_2Cl_2 (9:1, v/v) to afford compound **7** (4 mg). Fraction E11 (2.7 g) was fractionated by Sephadex® LH-20 CC and eluted with MeOH/CH_2Cl_2 (9:1, v/v) to afford 2 sub-fractions E11.1-E11.2. Fraction E11.2 (1 g) was chromatographed on silica gel CC eluting with CH_2Cl_2/MeOH (9:1, v/v) to afford four sub-fractions E11.2.1-E11.2.4. Fraction E11.2.1 (70 mg) was purified by Sephadex® LH-20 CC and eluted with MeOH/CH_2Cl_2 (9:1, v/v) to yield compound **4** (5 mg). Fraction E11.2.4 (125 mg) was purified by Sephadex® LH-20 CC and eluted with MeOH to yield compound **5** (6 mg).

Fraction E14 (2 g) was separated by Sephadex® LH-20 CC using MeOH as eluent to afford 3 sub-fractions E14.1-E14.3. Fraction E14.2 (0.2 g) was purified on silica gel CC eluting with CH_2Cl_2/MeOH (9:1, v/v) to afford compound **6** (12.5 mg).

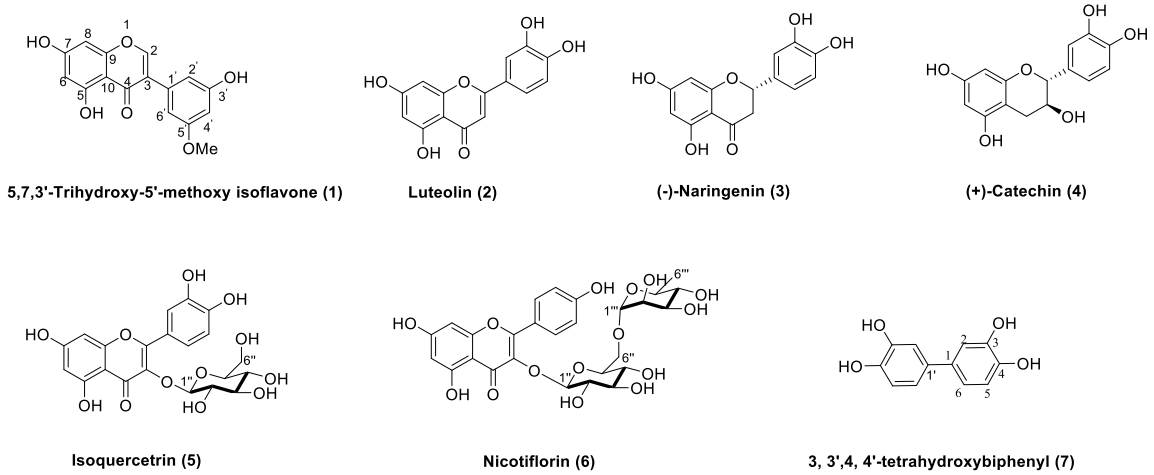

Figure 1. Chemical structures of isolated compounds **1-7**.

5,7,3′-Trihydroxy-5′-methoxy isoflavone (**1**) Yellow solid. ESI-MS *m/z*: 301 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 8.08 (1H, s, H-2), 7.07 (1H, s, H-4'), 7.00 (2H, s, H-2′, 6′), 6.36 (1H, d, *J* = 2.0 Hz, H-8), 6.24 (1H, d, *J* = 2.0 Hz, H-6), 3.90 (3H, s, 5'-OMe). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 179.8 (C-4), 166.0 (C-7), 163.8 (C-5), 159.7 (C-9), 155.0 (C-2), 149.2 (C-5′), 147.4 (C-3′), 125.2 (C-3), 124.5 (C-1′), 121.6 (C-6′), 117.3 (C-4′), 112.7 (C-2′), 106.2 (C-10), 100.2 (C-6), 94.9 (C-8), 56.4 (OMe).

Luteolin (2) Yellow solid; ESI-MS *m/z:* 287 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 7.41 (1H, d, *J* = 8.5 Hz, H-6′), 7.40 (1H, s, H-2′), 6.93 (1H, d, *J* = 8.5 Hz, H-5′), 6.56 (1H, s, H-3), 6.46 (1H, d, *J* = 2.0 Hz, H-8), 6.23 (1H, d, *J* = 2.0 Hz, H-6). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 183.8 (C-4), 166.3 (C-7), 166.1 (C-2), 163.2 (C-5), 159.4 (C-9), 151.0 (C-4′), 147.0 (C-3′), 123.7 (C-1′), 120.3 (C-6′), 116.7 (C-5′), 114.1 (C-2′), 105.3 (C-10), 103.9 (C-3), 100.1 (C-6), 95.0 (C-8).

(-)-Narigenin (3) Pale yellow solid; $[\alpha]^{25}_D$ -20.7 (*c* 0.2, MeOH). ESI-MS *m/z*: 289 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 7.34 (2H, d, *J* = 8.5 Hz, H-2′, 6′), 6.84 (2H, d, *J* = 8.5 Hz, H-3′, 5′), 5.91 (1H, s, H-8), 5.90 (1H, s, H-6), 5.37 (1H, dd, *J* = 13.0, 2.5 Hz, H-2), 3.13 (1H, dd, *J* = 13.0, 17.0 Hz, H-3a), 2.72 (1H, dd, *J* = 17.0, 2.5 Hz, H-3b). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 197.7 (C-4), 167.9 (C-7), 165.4 (C-5), 164.9 (C-9), 159.0 (C-4′), 131.1 (C-1′), 129.0 (C-2′, 6′), 116.3 (C-3′, 5′), 103.3 (C-10), 97.1 (C-6), 96.2 (C-8), 80.4 (C-2), 44.0 (C-3).

(+)-Catechin (**4**) White solid, $[\alpha]^{25}_D$ +15.5 (*c* 0.2, MeOH), ESI-MS *m/z*: 291 $[M + H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 6.86 (1H, d, *J* = 2.0 Hz, H-2′), 6.79 (1H, d, *J* = 8.0 Hz, H-5′), 6.74 (1H, dd, *J* = 8.0, 2.0 Hz, H-6′), 5.95 (1H, d, *J* = 2.5 Hz, H-8), 5.88 (1H, d, *J* = 2.5 Hz, H-6), 4.59 (1H, d, *J* = 7.5 Hz, H-2), 4.01 (1H, m, H-3), 2.87 (1H, dd, *J* = 16.0, 5.5 Hz, H-4a), 2.53 (1H, dd, *J* = 16.0, 8.0 Hz, H-4b). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 157.8 (C-7), 157.5 (C-5), 156.9 (C-9), 146.2 (C-3′), 146.1 (C-4′), 132.1 (C-1′), 120.0 (C-6′), 116.1 (C-5′), 115.3 (C-2′), 100.8 (C-10), 96.3 (C-6), 95.5 (C-8), 82.8 (C-2), 68.8 (C-3), 28.4 (C-4).

Isoquercetrin (**5**) Yellow solid. ESI-MS *m/z* 465 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 7.73 (1H, d, *J* = 2.0 Hz, H-2'), 7.60 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 6.88 (1H, d, *J* = 8.5 Hz, H-5'), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.22 (1H, d, *J* = 2.0 Hz, H-6), 5.25 (1H, d, *J* = 7.5 Hz, H-1"), 3.74–3.24 (6H, m, H-2"-6"). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 178.2 (C-4), 164.7 (C-7), 161.7 (C-5), 157.4 (C-9), 157.2 (C-2), 148.5 (C-4'), 144.4 (C-3'), 134.2 (C-3), 121.5 (C-1'), 121.9 (C-6'), 116.1 (C-5'), 114.2 (C-2'), 104.3 (C-10), 102.9 (C-1"), 98.6 (C-6), 93.5 (C-8), 77.0 (C-3"), 76.8 (C-5"), 74.2 (C-2"), 69.9 (C-4"), 61.1 (C-6").

Nicotiflorin (6) Yellow solid, ESI-MS *m/z* 595 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 8.07 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.91(2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.41 (1H, d, *J* = 2.0 Hz, H-8), 6.22 (1H, d, *J* = 2.0 Hz, H-6), 5.14 (1H, d, *J* = 7.5 Hz, H-1"), 4.54 (1H, d, *J* = 1.0 Hz, H-1'"), 3.82 (1H, br d, *J* = 10.5 Hz, Hz, H-6"b), 3.66 (1H, dd, *J* = 3.5 Hz, 1.5 Hz, H-2'"), 3.54 (1H, dd, *J* = 9.5, 3.5 Hz, H-3'"), 3.35-3.51 (5H, m, H-2", H-3", H-6"a, H-5"), 3.29-3.32 (2H, m, H-4", H-4'"), 1.14 (3H, d, *J* = 6.5 Hz, H-6'"). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 179.3 (C-4), 166.1 (C-7), 163.0 (C-5), 161.5 (C-2), 159.5 (C-4'), 158.5 (C-9), 135.5 (C-3), 132.4 (C-2', C-6'), 122.8 (C-1'), 116.1 (C-3', C-5'), 105.5 (C-10), 104.6 (C-1"), 102.4 (C-1'"), 100.0 (C-6), 95.0 (C-8), 78.1 (C-3"), 77.2 (C-5"), 75.8 (C-2"), 73.9 (C-4'"), 72.3 (C-3'"), 72.1 (C-2'"), 71.5 (C-4"), 69.5 (C-5'"), 68.6 (C-6"), 17.9 (C-6'").

3,3',4,4'-Tetrahydroxybiphenyl (**7**) Yellow solid, ESI-MS *m/z* 219 $[M+H]^+$. ^{1}H-NMR (500 MHz, CD_3OD) δ (ppm): 6.97 (2H, d, *J* = 2.0 Hz, H-2, H-2'), 6.77 (2H, d, *J* = 8.0 Hz, H-5, H-5'), 6.85 (2H, dd, *J* = 2.0, 8.0 Hz, H-6, H-6'). ^{13}C-NMR (125 MHz, CD_3OD) δ (ppm): 146.3 (C-3, C-3'), 145.2 (C-4, C-4'), 134.8 (C-1, C-1'), 118.9 (C-6, C-6'), 116.5 (C-5, C-5'), 114.6 (C-2, C-2').

2.4. Cytotoxic assay

Cytotoxic activities of isolated metabolites against KB, HepG2, MCF7, and LU cancer cell lines were tested by the MTT assay, that was described in the previous paper [7]. The isolated compounds were diluted in dimethylsulfoxide (DMSO) at the following concentrations: 128, 32, 8, 2, and 0.5 μg/mL and used for the test. Ellipticine was used as a reference compound.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow solid. The ^{1}H-NMR spectrum of the compound **1** showed typical signals of an isoflavonoid with a singlet at δ_H 8.08 (1H, s, H-2); two meta aromatic protons at δ_H 6.36 (1H, d, *J* = 2.0 Hz, H-8), 6.24 (1H, d, *J* = 2.0 Hz, H-6), and three protons of a 1,3,5-substituted phenyl ring at δ_H 7.07 (1H, s, H-4'), 7.00 (2H, s, H-2′, 6′). Besides that, a methoxy group was found at δ_H 3.90 (3H, s). The ^{13}C-NMR spectrum showed sixteen carbon signals including a carbonyl signal at δ_C 179.8 (C-4), a methoxy group at δ_C 56.4 (OMe) and fourteen aromatic carbons from δ_C 166.0 to 94.9 ppm. The molecular formula of **1** is suggested as $C_{16}H_{12}O_6$ based on the protonated molecular ion *m/z* 301 $[M+H]^+$ in the ESI-MS spectrum and NMR data. By comparison of the NMR and MS spectral data with those reported [8], the structure of **1** is identified as 5,7,3′-trihydroxy-5′-methoxy isoflavone (Figure 1).

Compound **2** was isolated as a yellow solid. The ^{1}H-NMR spectrum of **2** showed characteristic signals of a flavonone with two meta-aromatic signals at δ_H 6.46 (1H, d, *J* = 2.0 Hz, H-8) and 6.23 (1H, d, *J* = 2.0 Hz, H-6), a singlet at δ_H 6.56 (1H, s, H-3) and three proton of an ABX system at δ_H 7.41 (1H, d, *J* = 8.5 Hz, H-6′), 7.40 (1H, s, H-2′), 6.93 (1H, d, *J* = 8.5 Hz, H-5′). The ^{13}C-NMR exhibited fifteen carbon signals of flavanone with carbonyl group signals at δ_C 183.8 (C-4), and fourteen aromatic carbons ranging from 166.3 to 95.0 ppm. The ESI-MS

spectrum exhibited a *pseudo*-molecular ion peak $[M+H]^+$ at *m/z* 287, corresponding to a molecular formula of $C_{15}H_{10}O_6$. Based on the above spectral evidences, **2** was determined as luteolin. The NMR data of **2** were in accordance with those published [9].

Compound **3** was isolated as a pale yellow solid. The NMR spectrum revealed signals of a flavanone structure with 2 *meta*-protons at δ_H 5.91 (1H, s, H-8), 5.90 (1H, s, H-6), three protons of an A_2B_2 system at δ_H 7.34 (2H, d, J = 8.5 Hz, H-2′, 6′), 6.84 (2H, d, J = 8.5 Hz, H-3′, 5′), an oxymethine signal at δ_H 5.37 (1H, dd, J = 13.0, 2.5 Hz, H-2) and a methylene group at δ_H 3.13 (1H, dd, J = 13.0, 17.0 Hz, H-3a), 2.72 (1H, dd, J = 17.0, 2.5 Hz, H-3b). The ^{13}C-NMR showed fifteen carbon signals of a flavanone including a carbonyl carbon at δ_C 197.7 (C-4), twelve carbon signals at aromatic region, oxymethine carbon at δ_C 80.4 (C-2) and methylene group at δ_C 44.0 (C-3). The molecular formula of **3** was suggested as $C_{15}H_{12}O_6$ based on the pseudo molecular ion peak at *m/z* 289 $[M+H]^+$ in the ESI-MS spectrum and NMR data. Compound **3** was identified as (-)-narigenin (Figure 1) by comparison of NMR data with those published in literatures [10].

Compound **4** was obtained as a white solid. The ^{1}H-NMR spectrum showed the signals of a flavanonol structure with two meta protons at δ_H 5.95 (1H, d, J = 2.5 Hz, H-8), 5.88 (1H, d, J = 2.5 Hz, H-6), three protons of an ABX system at δ_H 6.86 (1H, d, J = 2.0 Hz, H-2′), 6.79 (1H, d, J = 8.0 Hz, H-5′), 6.74 (1H, dd, J = 8.0 Hz, 2.0 Hz, H-6′), two oxymethine signals at δ_H 4.59 (1H, d, J = 7.5 Hz, H-2) and 4.01 (1H, m, H-3), and a methylene group at δ_H 2.87 (1H, dd, J = 16.0, 5.5 Hz, H-4a), 2.53 (1H, dd, J = 16.0, 8.0 Hz, H-4b). The ^{13}C-NMR spectrum showed 15 carbon signals including 12 aromatic carbons, and 3 signals of pyran ring at δ_C 82.8 (C-2), 68.8 (C-3), 28.5 (C-4). The large coupling constant (7.5 Hz) of H-2 and H-3 indicated that two protons are in the opposite orientation. The ESI-MS spectrum showed a protonated molecular ion peak *m/z* 291 $[M+H]^+$, corresponding to a molecular formula $C_{15}H_{14}O_6$. On the basis of analytical NMR, and optical data, **4** was assigned as (+)-catechin. The NMR data were in accordance with those reported in previous paper [11].

Compound **5** was isolated from EtOAc extract as a yellow solid. The ^{1}H-NMR spectrum showed the signals of a flavonoid glycoside with two *meta* protons at δ_H 6.40 (1H, d, J = 2.0 Hz, H-8), 6.22 (1H, d, J = 2.0 Hz, H-6), three protons of an ABX system at δ_H 7.73 (1H, d, J = 2.0 Hz, H-2'), 7.60 (1H, dd, J = 2.0, 8.5 Hz, H-6'), 6.88 (1H, d, J = 8.5 Hz, H-5'), an anomer proton at δ_H 5.25 (1H, d, J = 7.5 Hz, H-1") and six protons of a sugar at δ_H 3.74–3.24 (6H, m, H-2"-6"). The ^{13}C-NMR spectrum showed 21 carbon signals including fifteen carbons of a flavonol, and 6 signals of a sugar moiety δ_C 102.9 (C-1"), 98.6 (C-6), 93.5 (C-8), 77.0 (C-3"), 76.8 (C-5"), 74.2 (C-2"), 69.9 (C-4") and 61.1 (C-6"). The molecular formula of 5 was assigned as $C_{15}H_{12}O_6$ based on the *pseudo*-molecular ion peak *m/z* 465 $[M+H]^+$ in the ESI-MS spectrum. The coupling constant of anomer proton (J = 7.5 Hz) and ^{13}C NMR data suggested the sugar was *β*-glucose. HMBC spectrum showed the correlation of H-1 (δ_H 5.25) to C-3 (δ_C 134.2). Compound **5** was assigned as quercetin-3-*β*-glucose (Figure 1) by comparison of NMR data with those published in literature [12].

Compound **6** was obtained as a yellow solid. The ^{1}H-NMR spectrum also showed the signals of a flavonoid glycoside. The aglycon was identified as keampferol with signals of two *meta* protons at δ_H 6.41 (1H, d, J = 2.0 Hz, H-8), 6.22 (1H, d, J = 2.0 Hz, H-6), four protons of an A_2B_2 system at δ_H 8.07 (2H, d, J = 8.5 Hz, H-2', H-6'), 6.91(2H, d, J = 8.5 Hz, H-3', H-5'). Sugar part was observed with two anomer protons at δ_H 5.14 (1H, d, J = 7.5 Hz, H-1") and δ_H 4.54 (1H, d, J = 1.0 Hz, H-1'"), eleven protons in the range of 3.82-3.39 ppm and a methyl group at δ_H 1.14 (3H, d, J = 6.5 Hz, H-6'"). The ^{13}C-NMR spectrum showed 27 carbon signals including 15 carbons of kaempferol aglycon, and 12 signals of 2 sugar moieties. The ESI-MS

spectrum showed a protonated molecular ion peak *m/z* 595 $[M+H]^+$, corresponding to a molecular formula $C_{27}H_{30}O_{15}$. HMBC spectrum showed the correlations of H-1" (δ_H 5.14) to C-3 (δ_C 135.5) and H-1'" (δ_H 4.54) to C-6" (δ_C 68.6) (Figure 2). The first sugar was assigned as *β*-glucose while the second sugar was identified as *α*-rhamnose based on the coupling constants of anomer protons (*J* = 7.5 Hz and 1.0 Hz, respectively) and ^{13}C NMR data [13]. Compound **6** was determined as kaempferol-3-rutinose or nicotiflorin (Figure 1) [13].

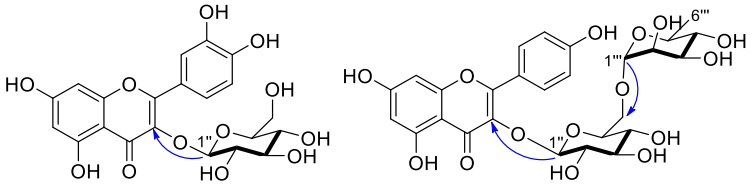

Figure 2. Key HMBC correlations of compound **5**-**6**.

Compound **7** was obtained as a brown solid. The ESI-MS spectrum showed a protonated molecular ion peak *m/z* 219 $[M+H]^+$. The ^{1}H-NMR spectrum showed the signals of three protons of an ABX system at δ_H 6.97 (d, *J* = 2.0 Hz), 6.77 (d, *J* = 8.0 Hz) and 6.85 (dd, *J* = 2.0, 8.0 Hz). The ^{13}C-NMR spectrum showed six carbon aromatic signals ranging from 146.3 to 114.6 ppm. Combined MS and NMR analysis, the molecular formula of **7** was suggested as $C_{12}H_{10}O_4$ and signals in ^{1}H and ^{13}C NMR spectra were duplicated peaks. Compound **7** was identified as 3,3',4,4'-tetrahydroxybiphenyl (Figure 1). The NMR data identical with those reported [14].

Compounds **3** and **6**-**7** were isolated from *Knema* genus for the first time while compounds **1**-**2** and **4**-**5** were isolated from *K. saxatilis* [15-16], *K. globularia* [17] and *K. laurina* [18]. Flavonoids compounds showed wide-range biological activities such as antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, anticancer and neuroprotective activity [19-22]. Compound 7 was found in ripe Pu-er tea [23] and reported to possess anti-inflammatory activity [24].

Table 1. Cytotoxic activity of isolated compounds **2** and **7**.

Compounds	**IC_{50} (μg/mL)**			
	KB	**HepG2**	**Lu**	**MCF7**
2	>128	>128	81.46 ± 3.26	>128
7	2.38 ± 0.11	18.65 ± 0.93	35.56 ± 1.17	18.25 ± 0.92
Ellipticine	0.50 ± 0.03	0.61 ± 0.03	0.47 ± 0.03	0.65 ± 0.04

The isolated compounds were evaluated for cytotoxicity against KB, HepG-2, Lu and MCF-7 cancer cell lines. In this assay, only biphenyl compound (**7**) showed strong cytotoxicity to KB cancer cell line with IC_{50} value of 2.38 ± 0.11 μg/mL. Compound **7** also displayed moderate activity against HepG2, Lu and MCF7 cancer cell lines. Other isolated flavonoids **1**-**6** were weakly active or inactive against tested cell lines.

4. CONCLUSIONS

Chemical investigation of the ethyl acetate extract of *K. pachycarpa* leaves led to the isolation of seven phenolic compounds including six flavonoids 5,7,3′-trihydroxy-5′-methoxy

isoflavone (**1**), luteolin (**2**),(-)-narigenin (**3**), (+)-catechin (**4**), isoquercetrin (**5**), nicotiflorin (**6**) and a biphenyl compound 3,3',4,4'-tetrahydroxybiphenyl (**7**). Compounds **3** and **6**-**7** were isolated from *Knema* genus for the first time. Biphenyl compound (**7**) showed strong cytotoxicity against KB cancer cell line with IC_{50} value of 2.38 ± 0.11 μg/mL and moderate activity against HepG-2, Lu and MCF-7 cancer cell lines.

Acknowledgements. The research funding from Vietnam National Foundation for Science and Technology Development (NAFOSTED) (Grant number: 104.01-2017.47) was acknowledged.

CRediT authorship contribution statement. CRediT authorship contribution statement. Tran Huu Giap, Vu Thi Hue,Nguyen Thi Hue and Nguyen Hoang Nam performed the experiments. Le Nguyen Thanh LN, Tran Huu Giap, Diep Thi Lan Phuong and Nguyen Quoc Vuong analyzed the data and wrote the article.

Declaration of competing interest. The authors declare that they have no conflict of interests.

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