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Journal of Medicinal Materials, 2022, Vol. 27, No. 2 (pp. 90 - 94)

PHENOLICS AND ALKALOIDS FROM THE FRUITS OF *PIPER LONGUM* L.

Nguyen Tra My[1], Nguyen Thi Thu[1], Vu Thi Diep[1], Hoang Thi Dieu Huong[1], Nguyen Thi Hang[1], Nguyen Thi My Linh[2], Vu Duc Loi[2], Do Thi Ha[1,*]

[1]National Institute of Medicinal Materials (NIMM), Hanoi, Vietnam;

[2]VNU University of Medicine and Pharmacy, Hanoi, Vietnam

*Corresponding author: hado.nimms@gmail.com

(Received April 05th, 2022)

Summary

Phenolics and Alkaloids from the Fruits of *Piper longum* L.

Seven compounds, including avenalumic acid methyl ester (**1**), methyl piperate (**2**), piperine (**3**), pipernonaline (**4**), (2*E*,4*Z*,8*E*)-*N*-[9-(3,4-methylenedioxyphenyl)-2,4,8-nonatrienoyl] piperidine (**5**), pipercide (**6**), and guineensine (**7**) were isolated from the ethyl acetate extract of *Piper longum* L. fruits. The structures of these compounds were identified by using extensive nuclear magnetic resonance spectroscopy (NMR) data, mass spectrometry (MS), and comparing with published literature. Compounds **1** and **6** were the first report from this species.

Keywords: *Piper longum, Fruit, Phenolics, Alkaloids, Piperine.*

1. Introduction

Piper longum L., a species of Piperaceae [1], distributes mainly in India, South China, Laos, Vietnam, and some other countries in Southeast Asia. In Vietnam, *P. longum* grows in mountainous and midland provinces, especially those with limestone mountains [2]. According to traditional medicine, *P. longum* is hot, pungent, and is often used to treat respiratory diseases such as sinusitis, pain in the nostrils, and nasal cavity… and digestive diseases such as abdominal pain, cold stomach causing vomiting, diarrhea, indigestion... [1],[2]. Studies on chemical composition in the *P. longum* fruits showed a large number of alkaloids and related compounds [3],[4]. In addition, *P. longum* contains lignans, essential oils, and organic acids [3],[4]. Biological investigation of extracts and compounds from *P. longum* fruits showed anti-cancer [5],[6], immunomodulation [7], antioxidant [8], hepatoprotection [8],[9], anti-inflammation [10],[11], and antibacterial effects [12]. Piperine, an alkaloid found in plants such as *P. nigrum* and *P. longum*, shows anti-lung cancer and metastatic activity. Piperlonguminin inhibits α-MSH-induced tyrosinase synthesis [5],[6]. Besides, *P. longum* extracts and piperinic acid decreased dose-dependently lympho cell ($CD4^+$ and $CD8^+$ cells) and cytokine levels in Balb/C mice [7]. To our knowledge, until now no report about the chemical composition of this species collected in Vietnam has been published. In this paper, we report the isolation and structural elucidation of phenolics and alkaloids compounds from the fruits of *P. longum*.

2. Materials and methods

2.1. Plant material

Samples of *Piper longum* L., Piperaceae were collected in Dak Song, Dak Nong, Vietnam in September 2020. The plant was identified by Assoc. Prof. Pham Thanh Huyen and MSc. Nguyen Van Hieu (National Institute of Medicinal Materials,

NIMM). A voucher specimen (DL-200420) was deposited in the Department of Medicinal Material Resources, NIMM (Hanoi, Vietnam).

2.2. General experimental procedures

NMR spectra were recorded on a Bruker Avance Digital 500 MHz NMR spectrometer (Karlsruhe, Germany) using TMS as the internal standard, *J* in Hz, at 294 K. ESI-MS data were analyzed *via* a Q Exactive Orbitrap Mass Spectrometer. *Silica gel* (Merck, 63–200 mm particle size), and YMC gel (75–150 μm) were used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* $60F_{254}$ and RP-$18F_{254}$ plates. Spots were detected under UV or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating.

2.3. Extraction and isolation

The dried fruits of *P. longum* (3.0 kg) were extracted and refluxed with 70% EtOH (3 times, 3 hours each time) at 80°C. The extracts were filtered, combined, and evaporated under reduced pressure, yielding a green residue (600.33 g), which was suspended in water and successively separated with *n*-hexane and ethyl acetate (EtOAc). The EtOAc extract (192.32 g) was separated on a *silica gel* CC with a gradient mixture of *n*-hexane-EtOAc (100:0-0:100, v/v) of increasing polarity as eluents to give ten fractions (1A-1K) and three compounds **2** (1.44 g), **3** (24.75 g), and **7** (0.48 g). Fraction 1I (5.03 g) was fractionated on RP-C_{18} gel CC eluting with an isocratic mixture of methanol-water (4:1, v/v) to obtain nine fractions (2A-2I) and two compounds **4** (0.86 g) and **6** (0.12 g). Fraction 2D (2.02 g) was applied to *silica gel* CC with an isocratic elution of *n*-hexane-EtOAc (4:1, v/v) to afford three fractions (3A-3C). Compound **5** (10 mg) was obtained from fraction 3C (56 mg) by using *silica gel* CC with an elution mixture of *n*-hexane-acetone (5:1, v/v). Fraction 1G (2.02 g) was applied to RP-C_{18} gel CC with an isocratic elution of methanol-water (3:2, v/v) to afford six fractions (6A-6F). Purification of fraction 6D (60.0 mg) by RP-C_{18} gel CC with a mixture of acetone-H_2O (3:2, v/v) to isolate compound **1** (10 mg).

Compound **1**: Pale yellow powder; ^{1}H-NMR (600 MHz, CD_3OD) δ_H: 5.97 (1H, d, J = 15.0 Hz, H-2), 7.45 (1H, dd, J = 10.8, 15.0 Hz, H-3), 6.84 (1H, dd, J = 10.8, 15.0 Hz, H-4), 6.93 (1H, d, J = 15.0 Hz, H-5), 7.40 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.80 (2H, d, J = 8.4 Hz, H-3′, H-5′), 3.76 (3H, s, OCH_3); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 169.6 (C-1), 119.6 (C-2), 147.4 (C-3), 124.4 (C-4), 142.5 (C-5), 129.1 (C-1′), 130.1 (C-2′, C-6′), 116.6 (C-3′, C-5′), 160.0 (C-4′), 51.9 (OCH_3).

Compound **2**: White powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 5.95 (1H, d, J = 15.6 Hz, H-2), 7.42 (1H, dd, J = 11.4, 15.6 Hz, H-3), 6.70 (1H, dd, J = 11.4, 15.0 Hz, H-4), 6.81 (1H, d, J = 15.0 Hz, H-5), 6.99 (1H, d, J = 1.8 Hz, H-2′), 6.78 (1H, d, J = 8.4 Hz, H-5′), 6.91 (1H, dd, J = 1.8, 8.4 Hz, H-6′), 5.98 (2H, s, OCH_2O), 3.76 (3H, s, OCH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 167.6 (C-1), 123.0 (C-2), 145.0 (C-3), 124.5 (C-4), 140.3 (C-5), 130.6 (C-1′), 105.9 (C-2′), 148.6 (C-3′), 148.3 (C-4′), 108.5 (C-5′), 120.0 (C-6′), 101.4 (OCH_2O), 51.5 (OCH_3); ESI-MS: *m/z* 233.10 $[M+H]^+$.

Compound **3**: Pale yellow powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 6.44 (1H, d, J = 15.0 Hz, H-2), 7.40 (1H, m, H-3), 6.73 (2H, m, H-4, H-5), 6.98 (1H, d, J = 1.8 Hz, H-2′), 6.78 (1H, d, J = 7.8 Hz, H-5′), 6.89 (1H, dd, J = 1.8, 7.8 Hz, H-6′), 5.97 (2H, s, OCH_2O), 3.58 (4H, br s, H-2″, H-6″), 1.58 (4H, m, H-3″, H-5″), 1.66 (2H, m, H-4″); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 165.5 (C-1), 120.1 (C-2), 142.5 (C-3), 125.4 (C-4), 138.2 (C-5), 131.1 (C-1′), 105.7 (C-2′), 148.2 (C-3′), 148.1 (C-4′), 108.5 (C-5′), 122.5 (C-6′), 101.3 (OCH_2O), 43.3 (C-2″), 26.7 (C-3″), 24.7 (C-4″), 25.6 (C-5″), 46.9 (C-6″); ESI-MS: *m/z* 286.2 $[M+H]^+$.

Compound **4**: White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H: 6.22 (1H, d, J = 15.0 Hz, H-2), 6.82 (1H, dt, J = 15.0, 7.0 Hz, H-3), 2.20 (4H, m, H-4, H-7), 1.67 (4H, m, H-5, H-6), 6.02 (1H, m, H-8), 6.27 (1H, d, J = 15.5 Hz, H-9), 6.88 (1H, d, J = 1.5 Hz, H-2′), 6.74 (2H, m, H-5′, H-6′), 3.48 (2H, br s, H-2″), 1.67 (6H, m, H-3″, H-4″, H-5″), 3.59 (2H, br s, H-6″), 5.92 (2H, s, OCH_2O); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: 165.6 (C-1), 120,5 (C-2), 145.6 (C-3), 32.7 (C-4), 29.0 (C-5), 29.7 (C-6), 32.4 (C-7), 128.9 (C-8), 129.6 (C-9), 132.4 (C-1′), 105.4 (C-2′), 146.6 (C-3′), 147.9 (C-4′), 108.2 (C-5′), 120.2 (C-6′), 100.9 (OCH_2O), 43.1 (C-2″), 25.6 (C-3″), 24.7 (C-4″), 26.6 (C-5″), 46.9 (C-6″); ESI-MS: *m/z* 343.3 $[M+H]^+$.

Compound **5**: White powder; ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H: 6.34 (1H, d, J = 15.0 Hz, H-2), 7.58 (1H, dd, J = 11.5, 14.5 Hz, H-3), 6.16 (1H, t, J = 11.5 Hz, H-4), 5.78 (1H, dt, J = 11.0, 8.0 Hz, H-5), 2.46 (2H, m, H-6), 2.29 (2H, m, H-7), 6.02 (1H, dt, J = 16.0, 7.0 Hz, H-8), 6.30 (1H, d, J = 16.0 Hz, H-9), 6.87 (1H, s, H-2′), 6.74 (2H, m, H-5′, H-6′), 5.93 (2H, s, OCH_2O), 3.49 (2H, br s, H-2″), 1.62 (6H, m, H-3″, H-4″, H-5″), 3.62 (2H, br s, H-6″); ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: 165.6 (C-1), 121.1 (C-2), 137.1 (C-3), 127.5 (C-4), 138.2 (C-5), 27.9 (C-6), 32.7 (C-7), 127.8 (C-8), 130.2 (C-9), 132.2 (C-1′), 105.5 (C-2′), 146.7 (C-3′), 147.9 (C-4′), 108.2 (C-5′), 120.4 (C-6′), 100.9 (OCH_2O), 43.2 (C-2″), 25.6 (C-3″), 24.6 (C-4″), 26.7 (C-5″), 46.9 (C-6″); ESI-MS: *m/z* 339.2 $[M-H]^-$.

Compound **6**: White powder; ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 5.95 (1H, d, J = 15.0 Hz, H-2), 7.13 (1H, dd, J = 11.0, 15.0 Hz, H-3), 6.22 (1H, dd, J = 11.0, 15.0 Hz, H-4), 1.51 (4H, m, H-7, H-8), 2.20 (4H, m, H-6, H-9), 6.10 (2H, m, H-5, H-10), 6.31

(1H, d, J = 16.0 Hz, H-11), 6.91 (1H, d, J = 2.0 Hz, H-2′), 6.73 (1H, d, J = 8.0 Hz, H-5′), 6.77 (1H, dd, J = 1.5, 8.0 Hz, H-6′), 5.92 (2H, s, OCH_2O), 3.08 (2H, d, J = 7.0 Hz, H-1″), 1.81 (1H, m, H-2″), 0.94 (6H, d, J = 6.5 Hz, H-3″, H-4″); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 169.2 (C-1), 123.1 (C-2), 142.1 (C-3), 129.7 (C-4), 143.8 (C-5), 33.8 (C-6), 30.1 (C-7), 29.7 (C-8), 33.7 (C-9), 129.9 (C-10), 131.0 (C-11), 133.8 (C-1′), 106.3 (C-2′), 149.4 (C-3′), 148.1 (C-4′), 109.0 (C-5′), 121.3 (C-6′), 102.2 (OCH_2O), 48.0 (C-1″), 29.5 (C-2″), 20.5 (C-3″, C-4″); ESI-MS: m/z 356.3 $[M+H]^+$.

Compound **7**: Pale yellow powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 5.75 (1H, d, J = 16.2 Hz, H-2), 7.18 (1H, dd, J= 10.8, 15.0 Hz, H-3), 6.12 (1H, dd, J= 10.8, 15.0 Hz, H-4), 6.08 (2H, m, H-5, H-12), 2.15 (4H, m, H-6, H-11), 1.37 (8H, m, H-7, H-8, H-9, H-10), 6.28 (1H, d, J = 15.6, H-13), 6.88 (1H, d, J = 1.2 Hz, H-2′), 6.74 (2H, m, H-5′, H-6′), 5.92 (2H, s, OCH_2O), 5.55 (1H, s, NH), 3.16 (2H, t, J = 13.2 Hz, H-1″), 1.76 (1H, m, H-2″), 0.92 (6H, d, J = 6.6 Hz, H-3″, H-4″); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 166.4 (C-1), 121.8 (C-2), 141.3 (C-3), 128.3 (C-4), 143.0 (C-5), 32.9 (C-6), 29.4 (C-7), 29.0 (C-8, C-9), 28.6 (C-10), 32.8 (C-11), 129.4 (C-12, C-13), 132.5 (C-1′), 105.4 (C-2′), 147.9 (C-3′), 146.6 (C-4′), 108.2 (C-5′), 120.2 (C-6′), 100.9 (OCH_2O), 47.0 (C-1″), 28.7 (C-2″), 20.1 (C-3″, C-4″); ESI-MS: m/z 382.3 $[M-H]^-$.

3. Results and discussion

Seven compounds were isolated from the 70% ethanol extract of the fruits of *P. longum* (**1**–**7**) (Fig. 1). From this extract, the identities of 7 compounds were verified by comparing their physicochemical and spectroscopic data to published values.

Compound **1** was isolated as pale-yellow powder. The ^{1}H-NMR of **1** showed signals of a *p*-hydroxybenzene structure at δ_H 7.40 (2H, d, J = 8.4 Hz, H-2′, H-6′) and 6.80 (2H, d, J = 8.4 Hz, H-3′, H-5′). Besides, **1** showed signals of two contiguous *trans*-double bonds at δ_H 5.97 (1H, d, J = 15.0 Hz, H-2), 7.45 (1H, dd, J= 10.8, 15.0 Hz, H-3), 6.84 (1H, dd, J= 10.8, 15.0 Hz, H-4) and 6.93 (1H, d, J = 15.0 Hz, H-5) and a methoxy group at δ_H 3.76 (3H, s, OCH_3). The ^{13}C-NMR showed signals of 12 carbon including two signals of the *p*-hydroxybenzene skeleton [δ_C 130.1 (C-2′, C-6′) and 116.6 (C-3′, C-5′)] and two aromatic carbon signals at δ_C 129.1 (C-1′) and 160.0 (C-4′). The four olefinic carbons were observed at δ_C 119.6 (C-2), 147.4 (C-3), 124.4 (C-4), and 142.5 (C-5). The signals at δ_C 169.6 and 51.9 are specific for a carboxyl carbon and a methoxy group, respectively. Based on spectral analysis, compound **1** was elucidated as methyl ester of avenalumic acid, also known as avenalumic acid methyl ester [13].

Compound **2** was obtained as a white powder and had the molecular formula $C_{13}H_{12}O_4$ which was identified by the analysis of its ESI-MS which gave a *quasi*-molecular ion at m/z 233.10 $[M+H]^+$. The ^{1}H-NMR spectrum of **2** also showed two *trans* double bonds [δ_H 5.95 (1H, d, J= 15.6 Hz, H-2), 7.42 (1H, dd, J = 11.4, 15.6 Hz, H-3), 6.70 (1H, dd, J = 11.4, 15.0 Hz, H-4) and 6.81 (1H, d, J = 15.0 Hz, H-5)] and a methoxy group [δ_H 3.76 (3H, s, OCH_3)] similar to **1**. However, **2** showed a 1,3,4-trisubstituted moiety with signals at δ_H 6.99 (1H, d, J = 1.8 Hz, H-2′), 6.78 (1H, d, J = 8.4 Hz, H-5′) and 6.91 (1H, dd, J = 1.8, 8.4 Hz, H-6′). In addition, **2** also showed a signal of the -OCH_2O- group at δ_H 5.98 (2H, s). The ^{13}C-NMR spectrum of **2** showed 13 carbon signals, including four olefinic carbon signals [δ_C 123.0 (C-2), 145.0 (C-3), 124.5 (C-4) and 140.3 (C-5)]; a methoxy signal (δ_C 51.5); a carboxyl signal (δ_C 167.6); six carbons in the ABX system [δ_C 130.6 (C-1′), 105.9 (C-2′), 148.6 (C-3′), 148.3 (C-4′), 108.5 (C-5′) and 120.0 (C-6′)] and a -OCH_2O- group (δ_C 101.4). Hence, the structure of **2** was elucidated as methyl piperate based on spectral analysis and by comparison with the reported literature [14].

Compound **3** was isolated as pale-yellow powder. The ESI-MS spectrum exhibited a *quasi*-molecular peak at m/z 286.2 $[M+H]^+$; combined with NMR data, it is suggested that the molecular formula of **3** is $C_{17}H_{19}NO_3$. The NMR spectra of **3** were similar to those of **2**. However, **3** did not show the signal of the methoxy group, instead of signals of a piperidine ring in the region of 1.58 - 3.58 ppm (10H). The ^{13}C-NMR spectrum of **3** showed signals of 17 carbons, including five carbons of the piperidine ring [δ_C 43.3 (C-2″), 26.7 (C-3″), 24.7 (C-4″), 25.6 (C-5″), and 46.9 (C-6″)]. The signal at δ_C 165.5 is specific for the amide carbonyl group. Comparing the NMR spectra [15], the structure of **3** was identified as piperine.

Compound **4** was obtained as a white powder. The molecular formula of **4** was suggested as $C_{21}H_{27}NO_3$ based on a *pseudo*-molecular peak at m/z 343.3 $[M+H]^+$ and NMR spectra. The MS spectrum showed that the molecular weight of **4** is more than **3** 56 u or 4 methylene groups. This was also confirmed by the NMR spectrum with the appearance of four methylene groups at δ_H 1.67 (4H, m), 2.20 (4H, m) and δ_C 29.0, 29.7, 32.4, and 32.7. Moreover, the NMR spectrum of **4** also showed signals of an amide group [δ_C 165.6 (C-1)], an ABX system (δ_H 6.88 (1H, d, J = 1.5 Hz, H-2′), 6.74 (2H, m, H-5′, H-6′), and a piperidine ring [δ_H 3.48 (2H, br s, H-2″), 1.67 (6H, m, H-3″, H-4″, H-5″), 3.59 (2H, br s, H-6″)]. Signals at δ_H 6.22 (1H, d, J = 15.0 Hz) and 6.27 (1H, d, J = 15.5 Hz) with peak splitting be predicted two *trans*-double bonds of **4** were located on either side of amide and ABX system and were confirmed based on NMR data and compared with the reported reference [16]. Based on the analysis

above, the structure of **4** was elucidated as pipernonaline [16].

Compound **5** was obtained as a white powder. Its molecular formula was suggested as $C_{21}H_{25}NO_3$ based on a negative ion peak at *m/z* 339.2 $[M-H]^-$ and NMR spectra. The NMR spectrum of **5** is similar to that of **4**, however, **5** appeared with one more *cis*-double bond at δ_H 6.16 (1H, t, J = 11.5 Hz, H-4), 5.78 (1H, dt, J = 8.0, 11.0 Hz, H-5) and δ_C 127.5 (C-4), 138.2 (C-5). In addition, **5** has only two methylene groups instead of four groups as in the structure of **4**. A methylene group of **4** was saturated to a double bond in the structure of **5**. Moreover, the configurations 2*E*, 4*Z*, and 8*E* were confirmed through the NOESY correlations between H-2 (δ_H 6.34) and H-4 (δ_H 6.16), between H-4 and H-5 (δ_H 5.78), between H-3 (δ_H 7.58) and H-6 (δ_H 2.46), and between H-7 (δ_H 2.29) and H-9 (δ_H 6.30). Thus, compound **5** was elucidated as (2*E*,4*Z*,8*E*)-*N*-[9-(3,4-methylenedioxyphenyl)-2,4,8-nonatrienoyl] piperidine by comparison of its NMR data with those published [17].

Compound **6** was obtained as a white powder. The molecular formula of **6** was found to be $C_{22}H_{29}NO_3$ via the NMR data and the *pseudo*-molecular ion peaks at *m/z* 356.3 $[M+H]^+$ and 354.25 $[M-H]^-$. The NMR spectra of **6** had similarities with **4** with the presence of a -OCH_2O- group; a 1,3,4-trisubstituted benzene ring and a *trans*-double bond attached to the benzene ring together with two *trans*-double bonds and four methylene groups in the carboxylic acid chain. However, the NMR spectrum of **6** did not show the signals of the piperidine ring, but instead the signals of $(CH_3)_2CHCH_2NH$- group at δ_H 3.08 (2H, d, J = 7.0 Hz, H-1″), 1.81 (1H, m, H-2″), 0.94 (6H, d, J = 6.5 Hz, H-3″, H-4) and δ_C 48.0 (C-1″), 29.5 (C-2″), and 20.5 (C-3″, C-4″). In addition, one more *trans*-double bond appeared in **6** at δ_H 6.22 (1H, dd, J = 11.0, 15.0 Hz, H-4), 6.10 (1H, m, H-5) and δ_C 129.7 (C-4), 143.8 (C-5). The positions of the *trans*-double bonds were confirmed based on NMR data and compared with published references [18]. Considering these observations in combination with reported data [18], compound **6** was determined to be pipercide (Fig. 1).

Compound **7** was obtained as a white powder. The ESI-MS spectrum of **7** showed a *pseudo*-molecular ion peak at *m/z* 382.3 $[M-H]^-$; combined with NMR data, it is indicated that the molecular formula of **7** is $C_{24}H_{33}NO_3$. The NMR spectra of **7** were similar to **6**; however, two more methylene groups appeared in the carboxylic acid chain. This was shown by the *pseudo*-molecular ion peak in ESI-MS (+28 u) and two ^{13}C-NMR signals (δ_C 28.6 and 29.0). Based on spectral analysis and by comparison with the reported literature [18], **7** was identified as guineensine (Fig. 1).

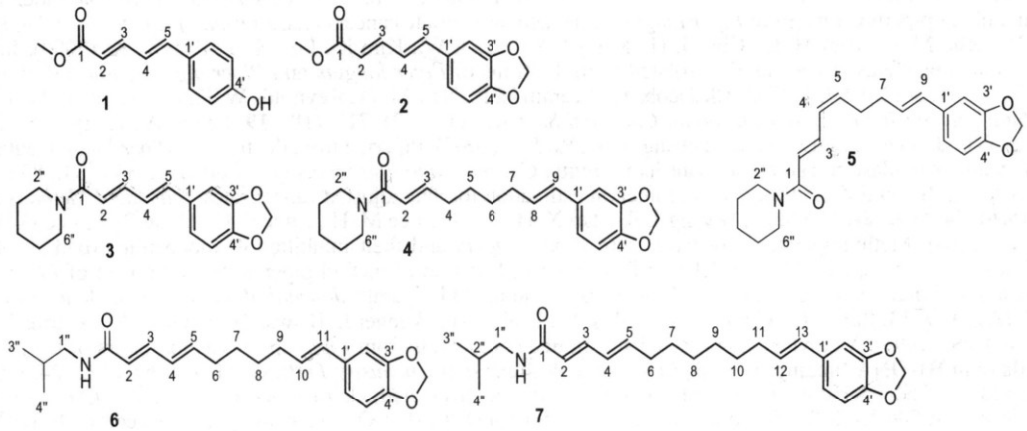

Fig. 1. Chemical structures of compounds (**1-7**) isolated from the fruits of *Piper longum*

Seven compounds including two phenolics (**1-2**) and five alkaloids (**3-7**) were isolated from *P. longum* fruit and the structures of these compounds were identified. Compounds **1** and **6** were first isolated from this species. The results of this study are consistent with the previous publications on *P. longum* [17],[19],[20],[21]... These compounds showed biological activities related to the activities of medicinal herbs. Methyl piperate (**2**) has antibacterial [22] and prevention of toxic carcinogens activities [23]; piperine (**3**) has anti-cancer [24] and antihyperlipidemic activities [25]; pipernonaline (**4**) has anti-cancer activity, especially against prostate cancer [26]; compound **5** showed anti-cell adhesion [27], antibacterial [17], anti-inflammation [28], and prevention of diabetes activities [17]; pipercide (**6**) has anti-larvae activity [29]; and anti-hepatitis virus [30], anti-tuberculosis [31], and antiparasitic [30] activities of guinensine (**7**) were reported. Avenalumic acid methyl ester (**1**) is isolated from *Poncirus trifoliata* [32] and there are currently not many pharmacological studies.

Through the isolation process, it can be seen that except for compounds **1** and **5** isolated with

small amounts, the remaining compounds have larger amounts. Therefore, it is possible to choose these compounds (**2-4**, **6**, and **7**) as an indicator in testing and evaluating the quality of the medicinal herb and medicinal products from the fruit of *P. longum*.

4. Conclusion

In this study, seven compounds, including avenalumic acid methyl ester (**1**), methyl piperate (**2**), piperine (**3**), pipernonaline (**4**), (2*E*,4*Z*,8*E*)-*N*-[9-(3,4-methylenedioxyphenyl)-2,4,8-nonatrienoyl] piperidine (**5**), pipercide (**6**), and guineensine (**7**) were isolated from the ethyl acetate extract of *P. longum* L.. Among them, compounds **1** and **6** were the first reported in *P. longum* L..

Acknowledgments: *This study was financially supported from the project*: **Study on anticancer and adjuvant cancer therapy drug from Vietnamese Traditional Medicine, NĐT.85.KR/20, Contract No. 09/2020/HĐ-NĐT signed 09/9/2020-MOST.**

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