SHORT REPORT

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Triterpenes from *Meliosma oldhamii* Miquel Branches and their Elastase Inhibitory Activities

Sang-Hee Byeon and Nam Ho Lee*

Department of Chemistry and Cosmetics, Jeju National University, Ara-1, Jeju 690-756, South Korea

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Abstract: Phytochemical investigation on the ethanol extracts of *Meliosma oldhamii* Miquel branches led to the isolation of seven triterpene constituents: betulin (**1**), lupeol (**2**), oleanolic acid (**3**), 3β-acetoxyolean-12-en-28-acid (**4**), 3β-acetoxyolean-12-en-28-aldehyde (**5**), 3β-acetoxy-28-hydroxyolean-12-ene (**6**) and maslinic acid (**7**). Their chemical structures were determined based on the spectroscopic studies, as well as by comparison with literature data. Elastase inhibition activities were examined for the isolates using ursolic acid as a positive control. In this test, the compounds **1** and **3** proved to inhibit porcine pancreatic elastase with an IC_{50} values of 39.3 and 39.5 μg/mL, indicating comparable activities to ursolic acid (IC_{50} = 28.5 μg/mL). This study demonstrated that the *M. oldhamii* extract including triterpenes has potentials applicable as anti-wrinkle ingredient in cosmetic formulations. All of the compounds **1**-**7** were isolated for the first time from *M. oldhamii*.

Keywords: *Meliosma oldhamii*; isolation; triterpenes; elastase inhibition.

1. Plant Source

As a part of our ongoing projects to find bioactive compounds from plants growing in Jeju Island [1-3], the ethanol extract of *Melosma oldhamii* Miquel was observed to have elastase inhibition activities. We herein describe the identification of the active constituents as well as their anti-elastase activities.

The plant *M. oldhamii* Miquel (Sibiaceae family) is a deciduous tree native to southern areas of Korea [4]. The branches of *M. oldhamii* were collected in September of 2009 from the Halla Botanical Garden in Jeju, located at the southernmost part of Korea. Identification of the plant was made by a botanist at the garden. A voucher specimen (sample number 179) was deposited at the Laboratory of Natural Product Chemistry, Department of Chemistry and Cosmetics, Jeju National University.

2. Previous Studies

In the screening of Korean medicinal plant extracts, *M. oldhamii* has been reported to present moderate α-glucosidase inhibitory activities [5]. However, as far as we know, phytochemical studies on this plant have not been reported yet.

* Corresponding author: E-mail: namho@jejunu.ac.kr (N. H. Lee), Phone +82-64-754-3548

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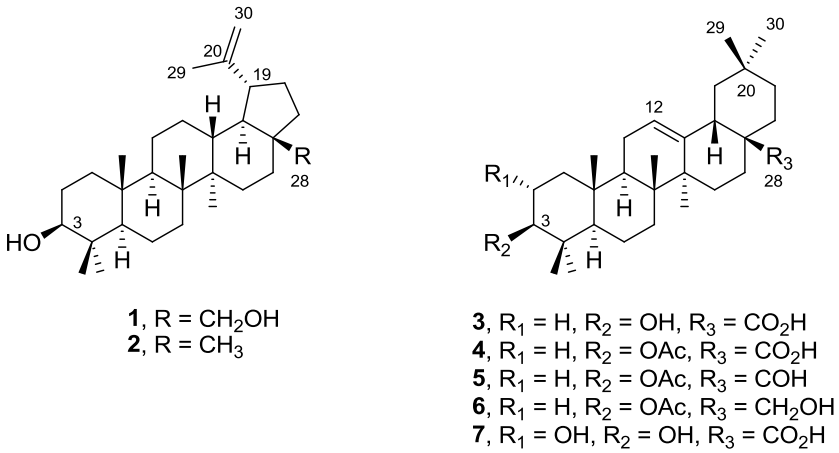
3. Present Study

Porcine pancreatic elastase inhibition activities were observed on the ethanol extract prepared from the branch parts of *M. oldhamii* (data not shown). In this experiment, the inhibitory activities were examined using N-Succ-$(Ala)_3$-*p*-nitroanilide (SANA) as the substrate by the spectrophotometric method. The product, *p*-nitroaniline, hydrolyzed by the action of elastase was monitored [6].

In order to identify the active constituents in the *M. oldhamii* extract, solvent fractionation was conducted to afford *n*-hexane, ethyl acetate (EtOAc), *n*-butanol and water-soluble portions. The EtOAc fraction exhibiting relatively good activities (data not shown) was selected and subjected to repeated column chromatography over silica gel and/or Sephadex LH-20. From these purification procedures, the compounds **1**-**7** were isolated (Figure 1).



1, R = CH_2OH
2, R = CH_3

3, R_1 = H, R_2 = OH, R_3 = CO_2H
4, R_1 = H, R_2 = OAc, R_3 = CO_2H
5, R_1 = H, R_2 = OAc, R_3 = COH
6, R_1 = H, R_2 = OAc, R_3 = CH_2OH
7, R_1 = OH, R_2 = OH, R_3 = CO_2H

Figure 1. Chemical structures of the isolated triterpenes **1**-**7** from *M. oldhamii*.

The compound **1** was determined to have 30 carbons including one olefin and six methyl groups based on ^{13}C and DEPT NMR spectra. All of the methyls were tertiary, which was indicated by singlet CH_3 signals in ^1H NMR spectrum. Th presence of vinyl olefin was recognized by the characteristic ^{13}C NMR signals at δ_C 150.6 (C-20) and 109.9 (C-30) along with proton resonance at δ_H 4.58 (1H) and 4.61 (1H) for H_2-30. Two oxygen-bearing carbons were observed at δ_C 79.1 (C-3) and 60.7 (C-28). Taken together, the compound **1** was identified as a triterpene, betulin [7]. The compound **2** exhibited very similar ^1H and ^{13}C NMR signals to those of the compound **1**. Compared to **1**, however, the compound **2** showed an additional methyl peak in place of betulin's oxymethylene (C-28) signal. Therefore, the compud **2** was determined to be a triterpene compound, lupeol [8]. By examination of NMR spectra, the compound **3** was also inferred a triterpene posessing 30 carbons with seven tertiary methyl groups. Carbon signals at δ_C 143.8 (C-13) and 122.8 (C-12) determined the presence of olefin unit. Signals for carbonyl at δ_C 180.8 (C-28) and oxymethyne at δ_C 79.2 (C-3) were characterized. Based on these data, the triterpene **3** was identified as oleanolic acid [9]. The compound **4** showed 32 carbon peaks whose δ_C patterns were very similar to those of oleanolic acid (**3**). Close inspection on the NMR data revealed that an additional acetyl group is attached at C-3. Therefore, the compound **4** was identified as 3β-acetoxyolean-12-en-28-acid [10]. Compared to **4**, the triterpene **5** showed an NMR resonace corresponding to aldehyde instead of carboxylic acid at C-28. Accordingly, the compound **5** was identified as 3β-acetoxyolean-12-en-28-aldehyde [10]. Likewise, the compound **6** was determined as 3β-acetoxy-28-hydroxyolean-12-ene [10]. The compound **7** exhibited 30 carbons including one olefin, two oxymethines and seven methyls on ^{13}C and DEPT NMR spectra. Compared to oleanolic acid (**3**), the compound **7** posessed a hydroxy group assigned at C-2 by examination of NMR spectra. Therefore, the compound **7** was identified as maslinic acid [11]. All of these structures were further confirmed by comparing the spectral data to those in literatures [7-11]. Even though all of the isolates **1**-**7** are know compounds, they were identified for the first time from *M. oldhamii*.

The skin is composed of three layers i.e. the epidermis, dermis and subcutaneous tissue. Elastin located in the dermis layer is matrix protein responsible for the skin elasticity. Damage to the elastin fiber leads to the decreased skin elasticity mainly seen in the aged skin [12]. Elastase is the serine protease enzyme capable of degrading elastin by hydrolysis of the peptide bonds. In this regard, elastase inhibitors have attracted attention as potential anti-aging ingredients in cosmetic preparations [13].

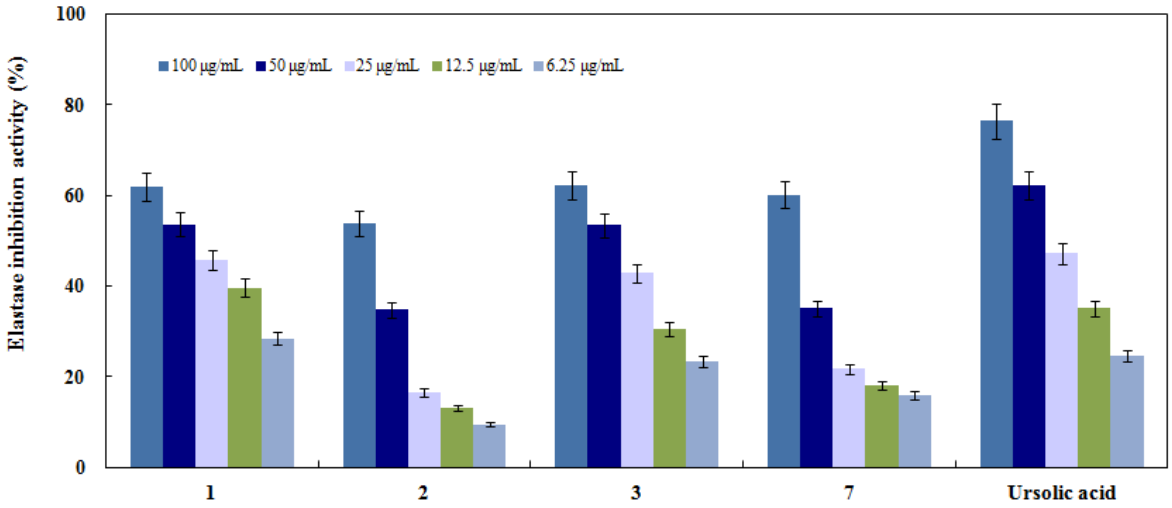


Figure 2. Elastase inhibition activities of the compounds **1**, **2**, **3** and **7**.

The anti-elastase properties for the isolates (**1-7**) were examined using N-succinyl-ala-ala-ala-*p*-nitroanalide (SANA) as the substrate. SANA is a chromogenic compound which releases a yellow color when cleaved by porcine panreatic elastase (PPE). The PPE and SANA were kept at concentrations of 100 μg/mL and 4 mM respectively, and the inhibitors was used at concentrations of 100, 50, 25, 12.5 and 6.25 μg/mL. The release of *p*-nitroaniline for 15 min at 25 ^{o}C was monitored by measuring the absorbance at 410 nm. Ursolic acid was used as a positive controp in this experiment. As shown in Figure 2, compounds **1**, **2**, **3** and **7** exhibited the elastase inhibition activities with dose dependent manner. The betulin (**1**) and oleanolic acid (**3**) inhibited porcine pancreatic elastase with an IC_{50} values of 39.3 and 39.5 μg/mL respectively, whose activities were comparable to ursolic acid (IC_{50} = 28.5 μg/mL). The lupeol (**2**) and maslinic acid (**7**) exhibited moderate activities with an IC_{50} 88.1 and 81.0 μg/mL respectively. The other compounds **4-6** showed little activities with IC_{50} over 100 μg/mL. It is interesting to recognize that the inactive compounds **4-6** have acetyl groups at C-3 position. From this observaton, it is obvious that the hydroxy group at C-3 in the triterpene is critical to exert elastase inhibition activity. Comparing the activities for the compounds **1** and **2**, it is suggested that the presence of polar functionality at C-28 is also important to gain higher activities.

In conclusion, phytochemical investigation of the branches of *M. oldhamii* led to the isolation of seven triterpene compounds. In the studies on the elastase inhibition for the isolates, the betulin (**1**) and oleanolic acid (**3**) exhibited relatively potent activities. These results demonstrated that the extract of *M. oldhamii* could be a candidate applicable as an anti-wrinkle agent by further study.

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