

## A LUPANE TRITERPENOID FROM THE AERIAL PARTS OF *ACANTHOPANAX TRIFOLIATUS*

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**Abstract:** *Acanthopanax trifoliatum* is a medicinal plant with high nutritional value because of the possesses ginseng-like activities. The phytochemical study on the aerial parts of *Acanthopanax trifoliatum* resulted in the discovery of a lupane-triterpene compound, betulinic acid (**1**). The chemical structure of **1** was elucidated by infrared (IR), mass (MS), nuclear magnetic resonance (NMR) spectroscopy as well as comparing with reported data.

**Keywords:** *Acanthopanax trifoliatum*; betulinic acid; NMR spectroscopy

### 1. Introduction

*Acanthopanax* species (Araliaceae) play an important role in traditional oriental medicine in Russia, Korea, Japan, China, and Vietnam. Its dried roots and stem barks are famous traditional medicine for treating rheumatism, arthritis, paralysis, sinew, and bone pain (Flora of China Editorial of Committee of Chinese Academy of Sciences, 1978). *Acanthopanax trifoliatum* (L.) MERR., which is called "ngũ gia bì gai" or "ngũ gia bì hương" in vietnamese, is distributed in Vietnam and used in the folk medicines as a drug with ginseng-like activities (Vo Van Chi, 1999; Pham Hoang Ho, 2000). It's also widely used as traditional oriental medicine having tonic, anti-rheumatic, longitudinal bone growth, adaptogenic activity, anti-fatigue, anti-stress, anti-ischemic heart disease benefits, tuberculosis, ulcer healing, tinea, and physical fitness (Perry Lily M., 1980). In recent, several phytochemical and pharmacological studies on *A. trifoliatum* have been carried out. Several kinds of chemical compositions have been reported, including triterpenoids, saponin, polyphenol, and flavonoids, fatty acids, etc...(Phan Van Kiem *et al*, 2003; Sithisarn P., 2014). In this study, we performed the isolation and identification of a betulinic acid (compound **1**) from the aerial parts of *A. trifoliatum*.

### 2. Experimental

#### 2.1. General experimental procedures

NMR spectra were recorded on Bruker Avance 500. ESI-MS: Agilent LC-MSD-Trap SL. TLC: Silica gel 60 F<sub>254</sub> (0.25mm, Merck); CC: Silica gel 60 (230-400 mesh, Merck) for

the first column, silica gel 60, 40-63  $\mu\text{m}$  (Merck), and Sephadex LH-20 for the following columns. Optical rotation was measured with a JASCO P2000 Polarimeter and D-line of the sodium spectrum. The IR spectra were recorded on a Jasco FTIR-4200 spectrophotometer (KBr).

#### 2.2. Plant material

The aerial parts of *Acanthopanax trifoliatum* were collected from the Quang Nam province in november 2020, and determined by Assoc. Prof. Do Huu Thu, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (NAF. 11.20.001) is deposited at Laboratory of Natural Products Research, Institute of Chemistry, VAST, Hanoi, Vietnam.

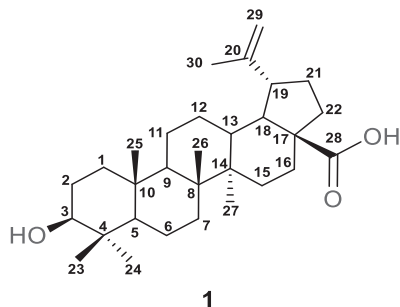
#### 2.3. Extraction and isolation

The aerial parts of *Acanthopanax trifoliatum* (3.1 kg) were chopped and extracted with MeOH (10 L) at room temperature repeated three times, three days for each time. The solvent was then removed under reduced pressure to yield a residue (427 g), then partitioned into the, n-hexane -soluble (20 g), CH<sub>2</sub>Cl<sub>2</sub>-soluble (42 g), EtOAc-soluble (69 g) and H<sub>2</sub>O-soluble parts (260 g). The CH<sub>2</sub>Cl<sub>2</sub>-soluble part (42 g) was subjected to column chromatography (CC) on silica gel eluting with a gradient of n-hexane–EtOAc 95:1 to 5:9) to yield twelve fractions (F1-F12). Fraction F4 (2.23 g) was subjected to CC on silica gel with n-hexane–EtOAc (10 : 1) to afford six fractions (F4.1–F4.6). Subfraction F4.3 (718 mg) was applied to silica gel column and eluted with a solvent system of n-hexane- EtOAc (3:1), and

then further purified on Sephadex LH-20 with  $\text{CH}_2\text{Cl}_2:\text{MeOH}$  (5:95) to obtain **1** (32 mg).

**Compound 1**, amorphous powder, m.p. 296–298°C, ESI-MS  $m/z$ : 479.35  $[\text{M}+\text{Na}]^+$ ,  $[\alpha]_{\text{D}}^{20} = -45.3$  ( $c = 0.1$ , MeOH), NMR spectra were recorded in methanol  $\text{d}_4$  (see table 1).

### 3. Results and discussion



**Compound 1** was obtained as a amorphous powder (32 mg), 296–298°C, and had a positive reaction in the Liebermann–Burchard test. The IR absorption bands of **1** displayed the presence of the hydroxyl group at  $3474\text{ cm}^{-1}$ , unsaturated carbonyl group at  $1684\text{ cm}^{-1}$ , and an alkenyl group at  $1643\text{ cm}^{-1}$ . The molecular formula was obtained as  $\text{C}_{30}\text{H}_{48}\text{O}_3$  with ESI-MS and with the observation of NMR spectra, the substance was suggested to be triterpenoid. The  $^{13}\text{C}$  NMR and DEPT spectra showed 30 carbon signals of a triterpene skeleton whose most of the carbon

signals resonated in the more shielding region from 15 to 58 ppm. The skeleton of **1** was recognized to be lupane triterpenoid with the typical alkenyl carbons at  $\delta_{\text{C}}$  152.0 (C-20) and 110.2 (C-29), six quaternary methyl carbons at  $\delta_{\text{C}}$  28.6, 19.5, 16.71, 17.65, 16.1 and 15.1. The spectral data also supported the presences of one carboxylic at  $\delta_{\text{C}}$  180.1 (C-28), one hydroxyl methine signal at  $\delta_{\text{C}}$  79.7 was assigned to C-3 as usual. The  $^1\text{H}$  NMR spectrum of **1** showed the presence of six tertiary methyl groups as singlets at  $\delta_{\text{H}}$  1.72 (H-30), 1.02 (H-27), 0.99 (H-26), 0.97 (H-24), 0.87 (H-23), 0.77 (H-28); two exo-methylene protons at  $\delta_{\text{H}}$  4.73 and 4.60. The *axial* position of the hydroxyl methine proton was confirmed by the signal at  $\delta_{\text{H}}$  3.14 (1H, dd, 11.5, 5.0), since the hydroxyl group at C-3 was determined as *equatorial* position ( $\beta$ -bond). As a result, compound **1** was established as betulinic acid (Satiraphan et al, 2012; Daisa Photis et al, 2017). Betulinic acid is a naturally occurring pentacyclic triterpenoid which has anti-malarial, and anti-inflammatory, anti-retroviral, anti-HIV properties, as well as a more recently discovered potential as an anticancer agent, by inhibition of *topoisomerase*.

**Table 1. NMR spectral data of compound 1**

Compound 1			
No	DEPT	$\delta_{\text{C}}$ (ppm)	$\delta_{\text{H}}$ (ppm)
1	$\text{CH}_2$	39.9	
2	$\text{CH}_2$	28.0	
3	CH	79.7	(1H, dd, 11.5, 5.0, H-3)
4	C	40.1	
5	CH	56.9	
6	$\text{CH}_2$	19.4	
7	$\text{CH}_2$	35.6	
8	C	41.9	
9	CH	52.0	
10	C	38.0	
11	$\text{CH}_2$	22.0	
12	$\text{CH}_2$	26.9	
13	CH	39.6	2.32 (1H, td, 13.0, 4.0, H-13)
14	C	43.6	
15	$\text{CH}_2$	31.7	
16	$\text{CH}_2$	33.4	2.24 (1H, dt, 13.0, 3.0, H-16a) 1.40 (1H, m, H-16b)
17	C	57.5	
18	CH	50.5	3.03 (1H, m, H-18)
19	CH	49.3	1.63 (1H, m, H-19)
20	C	152.0	
21	$\text{CH}_2$	30.8	
22	$\text{CH}_2$	38.1	1.93 (1H, m, H-22a) 1.44 (1H, m, H-22b)

23	CH <sub>3</sub>	28.6	0.87 (3H, s, H-23)
24	CH <sub>3</sub>	16.65	0.97 (3H, s, H-24)
25	CH <sub>3</sub>	16.1	0.77 (3H, s, H-25)
26	CH <sub>3</sub>	16.71	0.99 (3H, s, H-26)
27	CH <sub>3</sub>	15.1	1.02 (3H, s, H-27)
28	C=O	180.1	
29	=CH <sub>2</sub>	110.2	4.73 (1H, d, 2.0, H-29a), 4.60 (1H, m, H-29b)
30	CH <sub>3</sub>	19.5	1.72 (3H, s, H-30)

#### 4. Conclusion

Our results might contribute to further the knowledge about the circumscription of the species of *Acanthopanax* within the genus. Especially, further study can research into more species so that chemical data could be combined with morphological and genetic parameters.

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## MỘT TRITERPENOID KHUNG LUPANE TỪ CÁC BỘ PHẬN PHÍA TRÊN MẶT ĐẤT CỦA LOÀI *ACANTHOPANAX TRIFOLIATUS*.

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**Tóm tắt:** *Acanthopanax trifoliatum* là một cây thuốc nam có giá trị dinh dưỡng cao bởi vì nó có các hoạt tính tương tự như nhân sâm. Nghiên cứu hóa thực vật của các bộ phận ở phía trên mặt đất (thân, cành, lá, hoa, quả) của loài này, chúng tôi đã phân lập được một triterpene khung lupane đó là acid betulinic (**1**). Cấu trúc của chất **1** đã được làm sáng tỏ bằng việc kết hợp phân tích các phổ hồng ngoại, phổ khối lượng và phổ cộng hưởng từ hạt nhân.

**Từ khóa:** *Acanthopanax trifoliatum*; acid betulinic; phổ cộng hưởng từ hạt nhân (phổ NMR).