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# A New Pentacyclic Triterpenoid from *Albizia lebbekoides* (DC) Benth (Leguminosae)

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## ABSTRACT

*Albizia lebbekoides* (DC) Benth is one of the five species of the genus Albizia found in the Philippines. Not much is known about the chemistry and potential uses of this species. Solvent partition followed by sequential and repeated liquid chromatographic purification over silica led to the isolation of a compound from the hexane extract of *A. lebbekoides* leaves. A review of related literature and spectral analyses showed that the isolated compound is a new pentacyclic triterpenoid with an oleanane skeleton. Its structure was determined as 3β, 16α-dihydroxyolean-6(7),12(13)-dien-28-oic acid.

***Keywords:*** echinocystic acid; 3β, 16α-dihydroxyolean-6(7),12(13)-dien-28-oic acid; leaves

## INTRODUCTION

Albizia is a genus of about 150 species distributed throughout the world. They are mostly fast-growing subtropical and tropical trees and shrubs belonging to the subfamily *Mimosoideae* of the family Leguminosae. Five of them are found in the Philippines: *Albizia saman* (Jacq.) Merr., *Albizia lebbekoides* (DC) Benth, *A. lebbeck* (L.) Benth., *A. procera* (Roxb.) Benth., and *A. falcataria* (L.) Fosberg. *Albizia lebbekoides* (DC) Benth is commonly known as silk tree because its exotic flowers have delicate silky filaments and is locally known as "haluganit" (Tagalog), "maganhop-sa-bukid" (Bisaya), "lariskis" (Iloko) (https://uses.plantnet-project.org/en/Albizia_lebbekoides_(PROSEA)).

There is a dearth of studies on *A. lebbeckoides* and its synonyms *Mimosa carisquis* Blanco and *Pithecellobium myriophyllum* Gagnep (http://www.theplantlist.org/tpl/record/ild-46234). Most of the studies were done by a research group from Chulalongkorn University. They documented the α-glucosidase inhibitory activity (Tunsaringkarn et al., 2008; Tunsaringkarn et al., 2009), antioxidant activity (Ramli et al., 2008), inhibition of Heinz body induction (Tunsaringkarn et al., 2012), and stimulation of white blood cell proliferation (Tunsaringkarn et al., 2014) of *A. lebbekoides* extracts. Another study showed the antibacterial and antioxidant activities of the leaf

ethanol extract and the presence of alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, and glycosides (Hajrawati et al., 2019). This research aims to isolate and characterize a chemical compound from *A. lebbekoides* leaves that might add new knowledge to existing literature.

## METHODS

***Materials and Equipment.*** Vacuum liquid chromatography (VLC) and gravity column chromatography (GCC) were done using Merck silica gel particle size < 55μm and 0.063-0.200 mm, respectively. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 plates (Merck KGaA, Germany). TLC plates were visualized using UV light (Analytik Jena US), $I_2$ crystals, and vanillin-sulfuric acid followed by heating. All procedures were carried out at room temperature using distilled solvents purchased from commercial sources.

$^{1}H$ and $^{13}C$, DEPT, HSQC, HMBC, and DQF-COSY NMR spectra were recorded on a Bruker AVIII HD 600 NMR spectrometer (Scripps Research Institute USA). ESIMS was measured on an Agilent Q-TOF mass spectrometer in a positive-ion mode using an ESI ion source, with scan ranges ($m/z$) from 100 to 1000 (Scripps Research Institute USA). IR and UV spectra were recorded on a Nicolet Magna Infrared Spectrometer and UV-3101 PC double beam scanning spectrophotometer, respectively.

Leaves were collected from the University of the Philippines Diliman (UPD), Quezon City. A voucher specimen was submitted to the Jose Vera Santos Herbarium, Institute of Biology, UPD and was given accession number 14578.

***Extraction and Isolation.*** The dried leaves of *A. lebbekoides* (741.38 g) were homogenized and soaked in methanol (1kg:10 L). The methanol extract (5.8% yield) was filtered and concentrated *in vacuo*. The crude methanol extract was partitioned between water and hexane (1:6). The resultant dried hexane extract (3.7% yield) was subjected to VLC over a silica gel column (8 x 15.44 cm) and eluted with gradient mixtures of 500 mL hexane, hexane in 10% increments of ethyl acetate, ethyl acetate, ethyl acetate in 25% increments of ethanol, and ethanol under reduced pressure. Fractions were pooled by TLC analysis to yield 8 combined fractions.

Fraction 2 (0.74% yield) was chromatographed over a silica gel column (3.5 x 15 cm) and eluted with gradient mixtures of 300 mL hexane, hexane in 1% increments of ethyl acetate, ethyl acetate, ethyl acetate in 25% increments of ethanol, and ethanol. Test tubes with similar TLC profiles were pooled together and 10 sub-fractions were obtained. Sub-fraction 7 (0.132% yield) was purified by GCC over a silica gel column (2 x 41 cm), eluted with 200 mL hexane, hexane in 1% increments of ethyl acetate, and ethyl acetate, to yield a whitish isolate 7 (0.003% yield) with a single TLC spot with Rf 0.68 in 25% ethyl acetate in hexane as developing solvent: UV (MeOH) $\lambda_{max}$ 204.5 nm; IR (KBr disc) $\nu_{max}$ 3409, 2927, 2866, 1698, 1606, 1242, 1158, 1039, 955, 837 $cm^{-1}$; NMR data ($CDCl_3$), Table 1; positive ion HRESIMS m/z 447.3450

## RESULTS AND DISCUSSION

Sequential and repeated normal phase column chromatographic techniques led to the isolation of a TLC pure compound. The $^{13}C$ NMR spectrum (Table 1) showed 29 carbon resonances and comprising seven methyls, seven methylenes, eight methines and seven quaternary carbons. The IR spectrum showed the presence of C=O at 1698 $cm^{-1}$ but signals were absent in the NMR spectra.

**Table 1. NMR Spectral Data for Isolated Compound**

| C | $^{13}$C NMR (ppm)[1] | DEPT | HSQC[2] ($^{1}$H NMR) | HMBC ($^{1}$H NMR) |
|---|---|---|---|---|
| 1 | 39.6 | $CH_2$ | 1.24 | |
| 2 | 21.7 | $CH_2$ | 1.08 | |
| 3 | 71.1 | CH | 3.63, dd, J=12,6 Hz | |
| 4 | 43.4 | C | | 0.54, 1.01 |
| 5 | 56.0 | CH | | 0.54, 1.08 |
| 6 | 129.5 | CH | 5.02, dd, J=12,6 Hz | 5.14 |
| 7 | 138.3 | CH | 5.14 | 1.24 |
| 8 | 40.9 | C | | 1.52 |
| 9 | 51.3 | CH | 1.52 | 0.78, 1.03, 1.24, 5.14 |
| 10 | 23.1 | C | | 1.52 |
| 11 | 32.0 | $CH_2$ | 1.52 | |
| 12 | 117.6 | CH | 5.14 | |
| 13 | 139.7 | C | | |
| 14 | 34.3 | C | | |
| 15 | 49.5 | $CH_2$ | | |
| 16 | 71.2 | CH | 3.57 | 1.24 |
| 17 | 55.2 | C | | |
| 18 | 40.4 | CH | 2.02 | 0.84 |
| 19 | 37.2 | $CH_2$ | 1.72 | 0.78, 0.84 |
| 20 | 28.6 | C | | 0.84, 1.72 |
| 21 | 25.5 | $CH_2$ | 1.15, 1.38 | 0.84 |
| 22 | 38.1 | $CH_2$ | 1.24 | 0.78 |
| 23 | 12.2 | $CH_3$ | 0.54 | 1.01 |
| 24 | 13.2 | $CH_3$ | 1.01 | |
| 25 | 12.4 | $CH_3$ | 0.78 | |
| 26 | 21.5 | $CH_3$ | 1.03 | |
| 27 | 29.7 | $CH_3$ | 1.24 | |
| 29 | 19.1 | $CH_3$ | 0.78 | 0.84 |
| 30 | 21.2 | $CH_3$ | 0.84 | 0.78 |

[1] Data (δ) measured in $CDCl_3$ at 150 MHz and referenced to the solvent residual peak at ~δ 77
[2] Overlapped $^{1}$H NMR signals are reported without designated multiplicities

HRESIMS data showed a sodiated pseudo molecular ion peak at m/z 447.3450. The HRESIMS data is accounted for by [M-H+Na-COOH] (calcd 447.3238). The COOH proton at 10-12 ppm is usually very broad, often to the point of being hard to see above the baseline. The carboxyl carbon at 160-185 ppm is often weak because of nOe effects on other carbons in a proton-decoupled spectrum. In conjunction with the DEPT, HSQC, and IR data, a molecular formula of $C_{30}H_{46}O_4$ was deduced.

A pentacyclic system was inferred from eight degrees of unsaturation implied by the molecular formula and the unsaturated groups indicated by the NMR and IR spectroscopic data. Two oxygenated moieties were apparent at $\delta_C$ 71.1 (CH, $\delta_H$ 3.63) and $\delta_C$ 71.2 (CH, $\delta_H$ 3.57). The presence of a diene was inferred by the presence of signals at $\delta_C$ 117.6 (CH, $\delta_H$ 5.14), $\delta_C$ 129.5 (CH, $\delta_H$ 5.02), $\delta_C$ 138.3 (CH, $\delta_H$ 5.14) and $\delta_C$ 139.7 (C). An oleanane skeleton was deduced from the geminal methyl groups shown by HMBC cross peaks between $\delta_H$ 0.78 (H29) with $\delta_C$ 21.2 (C30) and $\delta_H$ 0.84 (H30) with $\delta_C$ 19.1 (C30) (Fig. 1). The structure of the E ring of oleanane skeleton was further established by the following HMBC cross peaks: $\delta_H$ 0.78 with $\delta_C$ 37.2 (C19), $\delta_C$ 38.1 (C22); $\delta_H$ 0.84 with $\delta_C$ 25.5 (C21), $\delta_C$ 28.6 (C20), $\delta_C$ 37.2 (C19), $\delta_C$ 40.4 (C18); $\delta_H$ 1.72 (H19) with $\delta_C$ 28.6 (C20); and $\delta_H$ 1.24 (C22) with $\delta_C$ 71.2 (C16-OH).

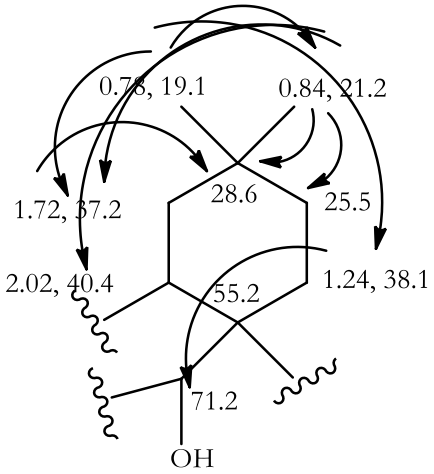


*Figure 1. HMBC Cross Peaks $^{1}H \rightarrow {}^{13}C$ of the E Ring of Oleanane*

The proposed structure of the isolated compound (Fig. 2) was supported by the HMBC correlations of H23 with C4 and C5; H2 with C5; H9/H11 with C10; and H1/H12/ H25/H26/H27 with C9. A diagnostic HMBC cross peak between H27 and C7 established the second double bond at C6-C7.

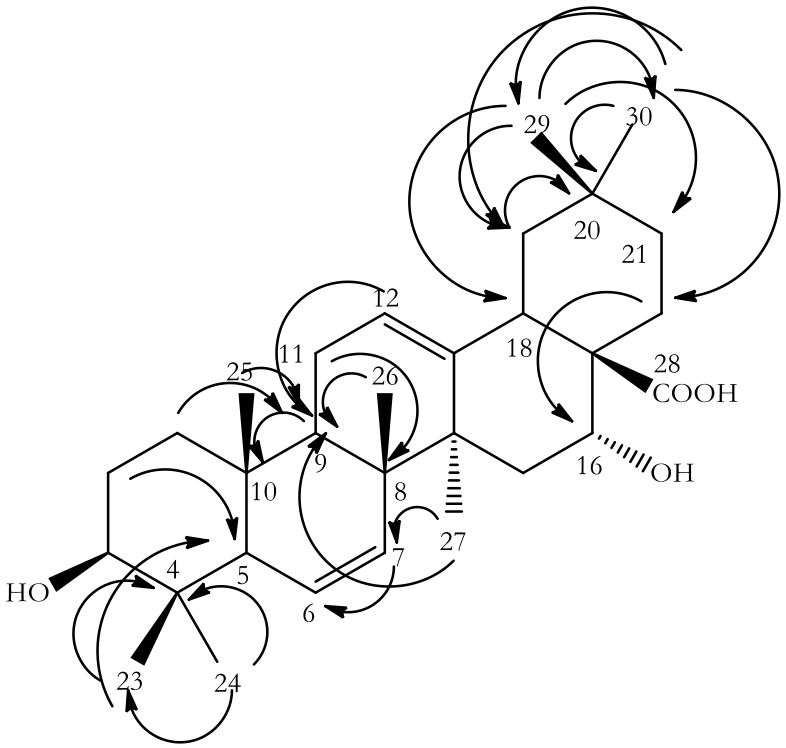


*Figure 2. HMBC Cross Peaks $^{1}H \rightarrow {}^{13}C$ of the Isolated Compound*

Oleanane triterpenoids are the largest group within the triterpenes with a huge number of biologically active compounds. They are structurally classified as olean-12-ene and 11-keto-olean-12-ene (Rios et al., 2000). The *Albizia* genus contained triterpenoid saponins, consisting mainly of glycosides of oleanolic acid (3β-hydroxyolean-12-en-28-oic acid), echinocystic acid (3β, 16α-dihydroxy-olean-12-en-28-oic acid), and acacic acid (3β, 16α, 21β-trihydroxy-12-en-28-oic acid) (Note et al., 2017). Glycosides of echinocystic acid have been isolated from *A. chevalieri*

(Note et al., 2017), *A. adianthifolia* (Haddad et al., 2004), *A. zygia* (Note et al., 2016), *A. grandibracteata* (Krief et al., 2005), *A. chinensis* (Liu et al., 2009), *A. gummifera* (Cao et al., 2007).

For oleananes with two double bonds, the positions of the double bonds are at C9-C11 and C12-C13 (Jayalakshmi et al, 2016; Sun et al., 2005); C11-C12 and C13-C18 (Zeng et al, 2015; Sun et al., 2005; Karliner and Djerassi, 1966); and C1-C2 and C12-C13 (Ren et al., 2015). This is the first report of an olean-12-ene triterpene with a second double bond at C6-C7.

## CONCLUSION

Normal phase chromatographic separation of the hexane extract from the leaves of *A. lebbekoides* yielded 3β, 16α-dihydroxyolean-6(7),12(13)-dien-28-oic acid, a new pentacyclic triterpene.

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