IJPSR (2012), Vol. 3, Issue 06 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 24 November, 2011; received in revised form 14 January, 2012; accepted 21 March, 2012

TRITERPENE ACIDS FROM n-HEHANE EXTRACT 0F *ALBIZZIA LEBBECK* BENTH.

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Keywords:
Albizzia lebbeck,
Leguminosae,
2α, 19α- dihydroxy – 3-oxo-12- ursen-28-oic acid,
2α, 16α, 19α- trihydroxy – 3-oxo-12-ursen- 28-oic acid

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ABSTRACT

Two triterpine acids like 2α, 19α- dihydroxy – 3-oxo-12- ursen- 28-oic acid (1), 2α, 16α, 19α- trihydroxy – 3-oxo-12- ursen- 28-oic acid (2) were isolated from the n-hexane soluble fractions of a methanol extract of the root of Albizzia lebbeck Benth. The structures of the isolated compounds were elucidated as by extensive spectroscopic studies, including high field NMR analyses. This is the first report of isolation of compounds 1- 2 from this species.

INTRODUCTION: *Albizzia lebbeck* Benth., Leguminosae (Bengali name- Shirish, Kalo koroi) is an unarmed deciduous tree of 12-21 m height that grows all over Bangladesh. The root of the plant has astringent property and is useful in opthalmia and skin diseases. The leaves are used in the treatment of night blindness and syphilis. The flowers of the plant are reputed for its aphrodisiac properties and are also useful in asthma and snake bite. The bark of the plant is anti-helmintic and used in the treatment of inflammation, bronchitis, toothache and leprosy [5, 9]. Previous phytochemical investigations with *A. lebbeck* revealed the occurrences of glycosides [11], alkaloids [2], terpenoids, steroids, saponins [8], anthraquinones and other phenolics [1]. We, herein, report the isolation of 2α, 19α- dihydroxy – 3-oxo-12- ursen- 28-oic acid (**1**), 2α, 16α, 19α- trihydroxy – 3-oxo-12- ursen- 28-oic acid (**2**).

MATERIALS AND METHODS:

General Experimental Procedures: The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument in CDCl3 and the δ values for were referenced to the residual nondeuterated solvent signal.

Plant Material: Root of A. lebbeck Benth. was collected from Dhaka in the month of September 2004. A voucher specimen for this collection has been maintained in Bangladesh National Herbarium (DACB 32758), Dhaka, Bangladesh.

Extraction and Isolation: The powdered root (1kg) of the plant was soaked in methanol (1.5L) for 16 days and then filtered through a cotton plug followed by Whatman filter paper no. 1. The extract was concentrated with a rotary evaporator and it afforded of the methanolic extract (20g). A portion (4g) of it was fractionated by the modified Kupchan partitioning protocol [6] into n-hexane, carbon tetrachloride, chloroform and aqueous soluble fractions. In brief, the methanolic extract (4g) was dissolved in 10% aqueous methanol (100mL) and extracted three times with n-hexane (300mL).

The remaining aqueous phase was then increased in polarity to 20% of water and extracted three times with carbon tetrachloride (300mL). The remaining aqueous phase was increased further in polarity to 40% of water and extracted three times with chloroform (300mL). Subsequent evaporation of solvents in open air dry the afforded crude extract were n-hexane (0.88g), carbon tetrachloride (0.50g), chloroform (1.06g) and aqueous soluble (1.40g). The n-hexane and chloroform soluble partitionates were separately chromatographed over silica gel (Kiesel gel 60, mesh 70-230) and both the columns were eluted with pet ether-ethyl acetate and ethyl acetate-methanol mixtures of increasing polarities. Compound 1 was isolated as colorless needles from the column fractions of the n-hexane partitionate eluted with 15% ethyl acetate in pet ether, while fractions eluted with 20% ethyl acetate upon re-chromatography over silica gel F254 provided compound 2 in pure form [7].

Elucidation:

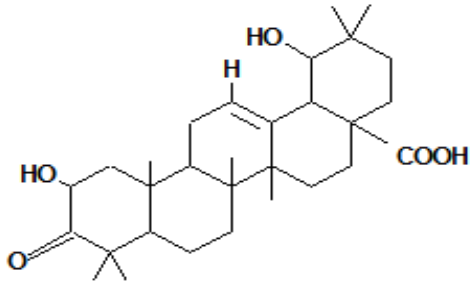
Compound-1: A colourless powder α! D +1.7°(c 1.0, EtOH); mp 215-217°C; calculated for C 30 H 46 O 5 : 486.368 ; ^{1}H-NMR (CDCl3 , 400 MHz): δ1.02 (3H, s, 24-H 3), 1.09 (s, 3H, 26-H 3), 1.10 (d, J = 4 Hz, 3H, 30-H 3), 1.15 (s, 3H, 25-H 3), 1.22 (s, 3H, 23-H 3), 1.24 (s, 1H, 5-H), 1.34 (s, 1H, 1-H a), 1.43 (s, 3H, 29-H 3), 1.58 (s, 1H, 20-H), 1.68 (s, 3H, 27-H 3), 1.90 (d, J = 8.6 Hz, 1H, 9-H), 2.07 (s, 1H, 21-H a), 2.10 (s, 1H, 22-H a), 2.33 (s, 1H, 21-H b), 2.41 (t, J = 6.2 Hz, 1H, 22-H b), 2.60 (d, J = 6.08 Hz, 1H, 1-H b), 3.21 (d, J = 10.4 Hz, 1H, 18-H), 4.00 (d, J =12.5 Hz, 1H, 2-H), 5.37 (br, s, 1H, 12-H).

Compound-2: A colourless powder, α D +12.0 (c 1.0, Pyridine); mp 270°C; calculated. for C 30 H 48 O6: 502.368; ^{1}H-NMR (CDCl3 , 400 MHz): δ 0.90 (s, 3H, 26-H 3), 1.02 (s, 3H, 23-H 3), 1.07 (s, 3H, 24-H 3), 1.14 (d, J = 4 Hz, 3H, 30-H 3), 1.24 (s, 3H, 25-H 3), 1.45 (s, 3H, 27-H 3), 1.81 (s, 3H, 29-H 3), 3.52 (s, 1H, 18-H), 3.76 (s, 1H, 2-H), 5.24 (br, s, 1H, 12-H).

RESULTS AND DISCUSSION: A total of two triterpine acids were isolated from n-hexane soluble fractions of a methanolic extract of the root bark of A. lebbeck Benth. by repeated chromatographic separation and purification over silica gel. The structures of the isolated compounds were solved by extensive NMR data analyses.

Compound-1: Compound -1 was obtained as a colourless powder. The 1 H-NMR spectrum showed the presence of a hydroxy methine proton at δ 4.00 (d, J = 12.5Hz, 1H), an olefinic proton at δ 5.37 (br, s, 1H), six singlets at δ 1.02 (3H), 1.09 (3H), 1.15 (3H), 1.22 (3H), 1.43 (3H), 1.68 (3H) for six tertiary methyl group, and a doublet at δ1.10 (d, J = 4 Hz, 3H) for a secondary methyl group. The secondary methyl signal on ring E provides a most useful indicator for the presence of an urs-12-ene skeleton. On the basis of the above spectral data and by comparison of these values with those reported for compound 1. Therefore the proposed structure of the compound-1 was established as 2α, 19α-Dihydroxy-3-oxo-12-ursen-28-oic ursen-28-oic acid 3,10.

Compound-2: Compound -2 was obtained as a colourless powder. The ^{1}H NMR spectra of 2 showed characteristic signals for the olefinic proton δ 5.24(br, s, H-12), δ 3.52 (s, H-18), and seven methyls including six singlets and one doublet (H3-30), consistent with a 19α-hydroxyursolic acid skeleton. In addition, the 1H NMR spectrum of 2 exhibited two mutually coupled carbonyl protons at δ 3.53 (d) and 5.09 (d). On the basis of the above spectral data and by comparison of these values with those reported for compound-2. Therefore the proposed structure of the compound-2 was established as 2α, 16α, 19α-Trihydroxy-3-Oxo-12-ursen-28-oic acid 3, 10.



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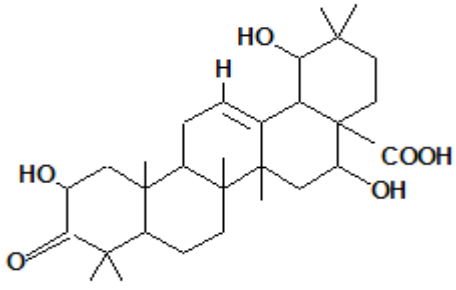

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CONCLUSION: The phytochemical study of the n-hexane and chloroform extractives of the A. lebbeck Benth. afforded four purified compounds, 2α, 19α-dihydroxy – 3-oxo-12- ursen- 28-oic acid (1), 2α, 16α, 19α- trihydroxy – 3-oxo-12- ursen- 28-oic acid (2) whose structures were established by extensive spectroscopic studies as well as comparison with published results. The bioactivities exhibited by the different extractives of A. lebbeck Benth. substantiate the folk uses of this plant species in various diseases.

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