

## Lupene Triterpenoids from the Stem Bark of *Albizia lebbeck*, Leaves of *Leptospermum scoparium* and Roots of *Nardostachys jatamansi*

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### Abstract

Lupene-type triterpenoids are used to treat arthritis, inflammation, cancer, diabetes, heart and renal disorders, hepatic toxicity and as anthelmintic, anti-bacterial, anticancer, anti-inflammatory, anti-malarial, antinociceptive, anti-HSV-1 and antioxidant drugs. These triterpenoids are distributed in many plants including *Albizia lebbeck* Benth. (Fabaceae), *Leptospermum scoparium* J. R. Forst. et G. Forst. (Myrtaceae) and *Nardostachys jatamansi* (D. Don) DC. (Valerianaceae). Elution of a methanolic extract of the stem bark of *A. lebbeck* on a silica gel column led to isolate betulinic acid derivatives characterized as lup-20(29)-en-2 $\beta$ -ol 3 $\beta$ -octanoyloxy-28-oic acid (3 $\beta$ -capryl 2 $\beta$ -hydroxybetulinic acid, **1**), lup-20(29)-en-2 $\beta$ -ol, 3 $\beta$ -decanoyloxy-28-oic acid (3 $\beta$ -caprioyl 2 $\beta$ -hydroxybetulinic acid, **2**) and lup-20(29)-en-2 $\beta$ -ol, 3 $\beta$ -octadec-9'-enoyloxy 28-oic acid (3 $\beta$ -oleyl 2 $\beta$ -hydroxybetulinic acid, **3**). Phytochemical investigation of a methanolic extract of the leaves of *L. scoparium* gave betulinic acid (**4**), morolic acid (**5**), 2 $\beta$ -acetoxo-3-acetyl morolic acid (**6**) and lup-20(29)-en-3 $\beta$ -ol-28-oic acid 3-O- $\alpha$ -L-xylopyranoside (betulinic acid 3 $\alpha$ -D-xylopyranoside, **7**). The methanolic extract of the roots of *N. jatamansi* on subjection to silica gel column furnished lup-20(29)-en-3 $\beta$ -olyl-3-O- $\beta$ -D-galactopyranosyl-2'-oleate (lupenyl 3 $\beta$ -O-galactopyranosyl oleate, **11**) together with 1-oleo-2,3-distreoglyceride (**8**), *n*-nonyl stearate (**9**) and stearyl stearate (**10**). The structures of these phytoconstituents have been established by spectral data analysis and chemical reactions.

**Keywords:** *Albizia lebbeck* Stem Bark; *Leptospermum scoparium* Leaves; *Nardostachys jatamansi* Roots; Lupenes; Isolation; Characterization

### Introduction

Triterpenes are important structural components of plant membranes and free triterpenes serve to stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membranes. Triterpenes are natural components of human diets. An average of 250 mg per day of triterpenes, largely derived from vegetables, plant oils, cereals and fruits is consumed. The intake of triterpenes reaches 400 mg/kg/day in Mediterranean countries if olive is taken. Lupeol is a pentacyclic triterpenol found in vegetables. It exhibits pharmacological activities against arthritis, inflammation, cancer, diabetes, heart diseases, renal disorders and hepatic toxicity [1-5]. Betulin, a constituent of the bark of *Betula* species, is a valuable component of cosmetic products used in hair conditioners and as an additive in the shampoo. Betulinic acid inhibits human immunodeficiency virus and shows anthelmintic, anti-bacterial, anticancer, anti-inflammatory, anti-malarial, antinociceptive, anti-HSV-1 and antioxidant activities. Lupene derivatives have an affinity for glucocorticoid receptors [6-8].

*Albizia lebbeck* Benth. syn. *Acacia lebbeck* (L.) Willd., *Acacia speciosa* (Jacq.) Willd., *Mimosa lebbeck* L. (Fabaceae), known as shirish, Indian walnut and fry wood, is a fast-growing, medium-sized deciduous tree is distributed in eastern Pakistan, India, Nepal, Bangladesh, Myanmar, Indonesia, Sri Lanka, Thailand and Australia [9]. The plant parts are used as anti-asthmatic, anti-inflammatory, anti-fertility, antiseptic, anti-tubercular and to treat asthma, cough, diarrhoea, dysentery, flu, genital diseases, gingivitis, gonorrhea, helminth infection, leprosy, leucorrhoea, lung problems, paralysis, allergic rhinitis, pectoral problems, ringworms, abdominal tumors and wounds [10]. Its root bark is effective to cure inflammation, gum and blood related diseases, leucoderma, itching, skin diseases, piles and bronchitis [9,11,12]. The roots possessed lupeol, steroids, 4-hydroxy-3-methoxycinnamic acid, trans-p-coumaric acid, echinocystic acid glycoside, fatty esters and acids, isotriacontanol, salicylic acid-2-O- $\beta$ -D-glucofuranosyl-6'-octadec-9'-enoate, lebbeksterone and n-tricontan-10 $\alpha$ -ol. The bark yielded flavonoids, lebbecadin, friedelin,  $\beta$ -sitosterol, acacic acid lactone 3-O-glycoside and anthraquinone glycosides. The heartwood contained

melanoxetin, d-pinitol, okanin, leucopelargonidin, melacacidins and lebbecacidin. The leaves gave flavonoids, N-benzoyl-L-phenylalaninol, friedelan-3-one, steroids, albigenin, albizziahexoside and cardiac glycoside. The seeds yielded budmunchiamines [13-19].

*Leptospermum scoparium* J. R. Forst. et G. Forst., syn. *Melaleuca scoparia* (J. R. Forst. et G. Forst.) L.f. (Myrtaceae), known as manuka, tea tree, and New Zealand tea tree. is a shrub or small tree native to New Zealand and Australia. It is grown as an ornamental garden plant in the United States, Britain, South Africa, Madeira and southern India in Tamil Nadu [20]. The plant is an effective antiseptic and used to treat respiratory system diseases, catarrh, bronchitis, coughs, colds, asthma, whooping cough, pneumonia, cystitis, urethritis, colitis, food toxicity, gastritis, vaginitis, leucorrhoea, gum infections, mouth ulcers, sore throats, tonsillitis and laryngitis. A leaf decoction is drunk to relieve urinary complaints and to reduce fever. The leaves are boiled in water and inhaled to alleviate asthma, bronchitis, colds, blocked sinuses and hay fever. The leaves and bark are boiled together, and the warm liquid is rubbed on the stiff backs and rheumatic joints. A leaf paste is applied to cure skin diseases and wounds. Its young shoots were chewed and swallowed for dysentery. The fresh, pungent leaves are taken as a fragrant and refreshing tea substitute [21]. The leaf essential oil is antiseptic, antifungal, anti-acne, anti-inflammatory, antihistamine, and anti-allergenic, cicatrizing, cytophylactic, deodorant and nervous relaxant; used to subside abscesses, arthritis, blisters, boils, bunions, chickenpox, corns, cuts, dandruff, eczema, herpes, impetigo, insect bites and stings, muscle aches, nail infections, scabies, ringworm, sores, varicose ulcers and warts [22,23]. The plant contained 5-methoxy-7-hydroxy-6,8-dimethylflavone, 5-hydroxy-6-methyl-7-methoxyflavone, 5,7-dimethoxy-6-methylflavone, uvaol and betulinol. The manuka essential oil consisted of cubebene/copaene, elemene, gurjunene/ aromadendrene, farnesene/ caryophyllene, selinene, (-)-trans-calamenene,  $\delta$ -cadinene, cadina-3,5-diene,  $\alpha$ -copaene, cadina-1,4-diene, oxy-sesquiterpenes,  $\alpha$ -pinene, myrcene, 1,8-cineole, linalool and triketones, viz., leptospermone, flavesone, and isoleptospermone [24-28]. The manuka honey possessed 4-hydroxybenzoic acid, dehydrovomifoliol, benzoic acid, kojic acid, 2-methoxybenzoic acid, syringic acid, 4-methoxyphenyllactic acid and methyl syringate [29].

*Nardostachys jatamansi* (D. Don) DC., syn. *N. chinensis* Batalin, *N. grandiflora* DC. (Valerianaceae), commonly known as jatamansi, Indian nard, balchar, sambul lateeb or spikenard, is a small, peren-

nial, rhizomatous herb which grows in steep, moist, rocky, undisturbed grassy rocky hillsides of northern India, Sikkim, Nepal, China, Tibet and Bhutan between 2200 - 5000 m above sea level [30-32]. Its rhizome is antimalarial, antiseptic, antispasmodic, appetizer, aromatic, carminative, diuretic, febrifuge, emmenagogue, expectorant, laxative, sedative, stimulant, stomachic, bitter tonic, tranquillizer and vermifuge. It is used to treat Alzheimer's disease, cold, colic, convulsions, cough, depressant, diabetes, diarrhoea, digestive and respiratory disorders, dysmenorrhoea, epilepsy, erysipelas, flatulence, hair loss, headache, hypertension, hysteria, insomnia, leprosy, memory disorders, neurosis and palpitation of heart [11,33,34]. A rhizome extract is taken as a remedy for fits and heart palpitations, to regulate constipation, urination, menstruation and digestion and to improve learning and memory [35]. Jatamansi extract is used as a hair tonic and to promote hair blackness, growth and luster [36]. A rhizome paste is applied to improve complexion of the skin. Jatamansi contained sesquiterpenes  $\alpha$ - and  $\beta$ -gurjunenes,  $\beta$ -maaliene, ledene oxide, allyl ionone valeranone, jatamansone, calarene,  $\alpha$ -cadinol and carveol, aliphatic constituents, xanthogalin, actinidine, nardal, jatamansin,  $\beta$ -sitosterol, iridoid, spirozatamole, jatamoles, nardostachysin, nardostachone, calarenol, pyranocoumarin, valerenic and palmitic acids and valerenyl isovalerate [36-43].

This manuscript describes isolation and characterization of lupene-type triterpenoids from the stem bark of *A. lebbeck*, leaves of *L. scoparium* and roots of *N. jatamansi*.

## Materials and Methods

### General Procedures

Melting points were determined on a Perfit apparatus without correction. The IR spectra were measured in KBr pellet on a Bio-Red FT-IR spectrometer. Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer. The  $^1\text{H}$  (400 MHz),  $^{13}\text{C}$  (100 MHz), COSY and HMBC NMR spectra were recorded on Bruker spectropin DRX spectrometer using  $\text{CDCl}_3$  or  $\text{DMSO-d}_6$  (Sigma-Aldrich, Bangalore, India) as a solvent and TMS as an internal standard. ESI MS analyses were performed on a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) mass spectrometer. Column chromatography separations were carried out on silica gel (Merck, 60 - 120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60  $\text{F}_{254}$ ) were used for analytical thin layer chromatography. Spots were visualized by exposing to iodine vapours and UV radiation and spraying with ceric sulphate solution.

## Plant Materials

The stem bark of *A. lebbeck* was collected from Allahabad, Uttar Pradesh and identified in the Faculty of Health Sciences, SHI-ATS, Allahabad. The leaves of *L. spermum* were obtained from the Pulney Hills (Western Ghats), Tamil Nadu and identified by Dr. M. P. Sharma, Department of Botany, Jamia Hamdard, New Delhi. The roots of *N. jatamansi* were procured from a local market of Delhi and identified by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi. The voucher specimens of these drugs were deposited in the herbarium of Phytochemistry Research Laboratory, Jamia Hamdard, New Delhi.

## Extraction and Isolation

Each 1 kg of stem bark of *A. lebbeck*, leaves of *L. spermum* and roots of *N. jatamansi* were coarsely powdered and extracted exhaustively separately with methanol in a Soxhlet apparatus. The extracts were concentrated under reduced pressure to get dark brown masses, 103.2 g, 126.8 g and 112.4 g, respectively.

The dried residue (100 g each) was dissolved in minimum amount of methanol and adsorbed on silica gel column grade (60 - 120 mesh) separately to obtain slurries. Each slurry was air-dried and chromatographed over silica gel columns loaded in petroleum ether individually. Each column was eluted with petroleum ether, petroleum ether -chloroform mixtures, chloroform and chloroform - methanol mixtures in order of increasing polarity to obtain the compounds.

### Isolation of Phytoconstituents from *A. lebbeck*

#### 3 $\beta$ -Capryl 2 $\beta$ -hydroxybetulinic acid (1)

Elution of the column of *A. lebbeck* extract with chloroform furnished colorless crystals of **1**, recrystallized from chloroform - methanol (1 : 1), 251 mg, m. p. 223 - 224 °C,  $[\alpha]_D^{20}$  - 3° (conc. 0.5 in methanol); UV  $\lambda_{max}$  (MeOH): 211 nm; IR  $_{\gamma_{max}}$  (KBr): 3466, 3281, 2924, 2851, 1721, 1686, 1635, 1462, 1376, 1193, 1107, 1033, 982, 721 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  4.68 (1H, br s, H<sub>2</sub> - 29a), 4.55 (1H, br s, H<sub>2</sub> - 29b), 4.22 (1 H, d, J = 5.2 Hz, H-3 $\alpha$ ), 3.72 (1 H, ddd, J = 4.6, 5.2, 8.9 Hz, H-2 $\alpha$ ), 2.26 (2 H, t, J = 6.8 Hz, H-2'), 1.64 (3H, br s, Me-30), 1.26 (12 H, brs, 6 x CH<sub>2</sub>), 1.23 (3H, br s, Me-23), 0.93 (3H, br s, Me-24), 0.86 (3H, br s, Me-25), 0.84 (3H, t, J = 6.5 Hz, Me-10'), 0.77 (3H, br s, Me-26), 0.65 (3H, br s, Me-27), 2.96 - 1.30 (27 H, m, 11 x CH<sub>2</sub>, 5 x CH); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  38.45 (C-1), 69.12 (C-2), 76.73 (C-3), 38.24 (C-4), 55.36 (C-5), 18.89 (C-6), 33.88 (C-7), 41.94 (C-8), 49.91 (C-9),

37.54 (C-10), 24.47 (C-11), 25.18 (C-12), 38.83 (C-13), 46.56 (C-14), 31.681 (C-15), 36.68 (C-16), 54.87 (C-17), 48.49 (C-18), 46.43 (C-19), 150.23 (C-20), 30.06 (C-21), 36.31 (C-22), 29.05 (C-23), 15.90 (C-24), 17.93 (C-25), 15.75 (C-26), 13.92 (C-27), 177.10 (C-28), 109.58 (C-29), 15.66 (C-30), 174.41 (C-1'), 33.62 (C-2'), 31.30 (C-3'), 29.17 (C-4'), 28.55 (C-5'), 28.04 (C-6'), 22.10 (C-7'), 14.33 (C-8'); ESI MS m/z (rel. int.): 598 [M]<sup>+</sup> (C<sub>38</sub>H<sub>62</sub>O<sub>5</sub>) (2.3), 471 (11.2), 455 (99.5), 127 (3.1).

#### 3 $\beta$ -Caprioyl 2 $\beta$ -hydroxybetulinic acid (2)

Further elution of the column with chloroform gave yellow amorphous powder of **2**, recrystallized from chloroform - methanol (1 : 1), 251 mg, m. p. 226 - 228°C,  $[\alpha]_D^{20}$  - 2° (conc. 0.5 in methanol); UV  $\lambda_{max}$  (MeOH): 211 nm; IR  $_{\gamma_{max}}$  (KBr): 3471, 3263, 2926, 2853, 1722, 1686, 1641, 1457, 1375, 1192, 1033, 982, 720 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  4.68 (1H, br s, H<sub>2</sub> - 29a), 4.58 (1H, br s, H<sub>2</sub> - 29b), 4.23 (1 H, d, J = 5.2 Hz, H-3 $\alpha$ ), 3.70 (1 H, ddd, J = 4.4, 5.2, 8.8 Hz, H-2 $\alpha$ ), 2.23 (2 H, t, J = 7.2 Hz, H<sub>2</sub> - 2'), 1.64 (3H, br s, Me-30), 1.26 (8 H, brs, 4 x CH<sub>2</sub>), 1.23 (3H, br s, Me-23), 0.91 (3H, br s, Me-24), 0.86 (3H, br s, Me-25), 0.84 (3H, t, J = 6.3 Hz, Me-8'), 0.76 (3H, br s, Me-26), 0.65 (3H, br s, Me-27), 2.98 - 1.01 (27 H, m, 11 x CH<sub>2</sub>, 5 x CH); <sup>13</sup>C NMR (CDCl<sub>3</sub>):  $\delta$  38.45 (C-1), 69.25 (C-2), 76.74 (C-3), 38.22 (C-4), 55.37 (C-5), 18.89 (C-6), 33.87 (C-7), 41.95 (C-8), 49.89 (C-9), 37.54 (C-10), 24.47 (C-11), 25.03 (C-12), 38.83 (C-13), 43.11 (C-14), 31.28 (C-15), 36.68 (C-16), 54.86 (C-17), 48.47 (C-18), 46.57 (C-19), 150.27 (C-20), 30.05 (C-21), 36.29 (C-22), 28.53 (C-23), 15.90 (C-24), 17.92 (C-25), 15.68 (C-26), 13.92 (C-27), 177.20 (C-28), 109.61 (C-29), 20.41 (C-30), 174.26 (C-1'), 33.62 (C-2'), 31.66 (C-3'), 29.01 (C-4'), 28.70 (C-5'), 28.05 (C-6'), 27.12 (C-7'), 26.58 (C-8'), 22.08 (C-9'), 14.31 (C-10'); ESI MS m/z (rel. int.): 626 [M]<sup>+</sup> (C<sub>40</sub>H<sub>66</sub>O<sub>5</sub>) (2.1), 471 (13.8), 455 (98.6), 171 (38.3), 155 (1.2).

#### 3 $\beta$ -Oleiyl 2 $\beta$ -hydroxybetulinic acid (3)

Elution of the column with chloroform - methanol (49 : 1) yielded yellow amorphous powder of **3**, recrystallized from chloroform - methanol (1 : 1), 251 mg, m. p. 193 - 104°C,  $[\alpha]_D^{20}$  - 3° (conc. 0.5 in methanol); UV  $\lambda_{max}$  (MeOH): 211 nm; IR  $_{\gamma_{max}}$  (KBr): 3419, 3275, 2919, 2850, 1725, 1687, 1641, 1435, 1388, 1376, 1273, 1238, 1194, 1108, 1032, 883, 720 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  5.32 (1H, m, H - 9'), 5.30 (1H, m, H - 10'), 4.68 (1H, br s, H<sub>2</sub> - 29a), 4.55 (1H, br s, H<sub>2</sub> - 29b), 4.22 (1 H, d, J = 5.3 Hz, H-3 $\alpha$ ), 3.76 (1 H, ddd, J = 5.3, 5.5, 8.5 Hz, H-2 $\alpha$ ), 2.27 (2 H, t, J = 7.6 Hz, H<sub>2</sub> - 2'), 1.68 (3H, br s, Me-30), 1.25 (20 H, brs, 10 x CH<sub>2</sub>), 1.23 (3H, br s, Me-23), 0.92 (3H, br s,

Me-24), 0.87 (3H, br s, Me-25), 0.84 (3H, t,  $J = 6.5$  Hz, Me-18'), 0.77 (3H, br s, Me-26), 0.65 (3H, br s, Me-27), 2.96 - 1.30 (29 H, m, 12 x  $\text{CH}_2$ , 5 x CH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  38.43 (C-1), 69.75 (C-2), 76.73 (C-3), 38.23 (C-4), 55.39 (C-5), 18.77 (C-6), 33.88 (C-7), 41.92 (C-8), 49.91 (C-9), 37.52 (C-10), 24.43 (C-11), 25.08 (C-12), 39.04 (C-13), 46.58 (C-14), 31.67 (C-15), 36.64 (C-16), 54.82 (C-17), 48.51 (C-18), 46.55 (C-19), 150.23 (C-20), 30.08 (C-21), 36.31 (C-22), 29.04 (C-23), 15.75 (C-24), 17.98 (C-25), 15.61 (C-26), 13.96 (C-27), 177.16 (C-28), 109.58 (C-29), 20.43 (C-30), 174.44 (C-1'), 33.67 (C-2'), 28.92 (C-3'), 28.85 (C-4'), 28.75 (C-5'), 28.55 (C-6'), 28.04 (C-7'), 31.30 (C-8'), 129.66 (C-9'), 127.70 (C-10'), 30.89 (C-11'), 29.17 (C-12'), 29.17 (C-13'), 28.92 (C-14'), 27.12 (C-15'), 26.58 (C-16'), 22.09 (C-17'), 14.33 (C-18'); ESI MS  $m/z$  (rel. int.): 736  $[\text{M}]^+$  ( $\text{C}_{48}\text{H}_{80}\text{O}_5$ ) (12.6), 471 (57.5), 455 (98.6), 281 (8.4), 265 (8.7).

### Isolation of phytoconstituents from *L. scoparium*

#### Betulinic acid (4)

Elution of the column with chloroform - methanol (49 : 1) furnished colourless powder of **4**, recrystallized from chloroform - methanol (1 : 1), 143 mg, m. p. 316 - 318°C,  $[\alpha]_D^{20} + 7.8^\circ$  (conc. 0.9 in pyridine); UV  $\lambda_{\text{max}}$  (MeOH): 211 nm; IR  $_{\text{max}}$  (KBr): 3481, 3215, 2925, 2847, 1692, 1642, 1455, 1315, 1260, 1191, 1041, 883  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  4.67 (1H, s,  $\text{H}_2$  - 29a), 4.55 (1H, s,  $\text{H}_2$  - 29b), 3.12 (1 H, dd,  $J = 5.5, 9.0$  Hz, H-3 $\alpha$ ), 1.64 (3H, br s, Me-30), 1.19 (3H, br s, Me-23), 0.90 (3H, br s, Me-25), 0.88 (3H, br s, Me-26), 0.75 (3H, brs, Me-24), 0.69 (3H, br s, Me-27), 2.91 - 1.34 (25 H, m, 10 x  $\text{CH}_2$ , 5 x CH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  36.37 (C-1), 29.22 (C-2), 76.79 (C-3), 38.95 (C-4), 55.41 (C-5), 17.98 (C-6), 36.37 (C-7), 40.34 (C-8), 46.61 (C-9), 36.73 (C-10), 20.47 (C-11), 25.08 (C-12), 38.29 (C-13), 41.99 (C-14), 31.73 (C-15), 27.16 (C-16), 54.92 (C-17), 49.96 (C-18), 48.55 (C-19), 150.28 (C-20), 30.12 (C-21), 33.94 (C-22), 28.09 (C-23), 14.39 (C-24), 15.72 (C-25), 15.94 (C-26), 15.79 (C-27), 177.23 (C-28), 109.61 (C-29), 18.94 (C-30); ESI MS  $m/z$  (rel. int.): 456  $[\text{M}]^+$  ( $\text{C}_{30}\text{H}_{48}\text{O}_3$ ) (15.3), 438 (100).

#### Morolic acid (5)

Further elution of the column with chloroform - methanol (49 : 1) yielded colourless powder of **5**, recrystallized from chloroform - methanol (1 : 1), 211 mg, m. p. 297 - 298°C; UV  $\lambda_{\text{max}}$  (MeOH): 213 nm; IR  $_{\text{max}}$  (KBr): 3464, 3382, 2947, 2857, 1690, 1635, 1454, 1390, 1214, 1066  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  5.45 (1H, s, H - 19), 3.35 (1 H, dd,  $J = 4.5, 8.1$  Hz, H-3 $\alpha$ ), 0.86 (3H, br s, Me-23), 0.84 (3H, br s,

Me-29), 0.82 (3H, br s, Me-25), 0.80 (6H, br s, Me-24, Me-26), 0.79 (3H, brs, Me-30), 0.76 (3H, br s, Me-27), 2.91 - 1.34 (23 H, m, 10 x  $\text{CH}_2$ , 3 x CH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  41.40 (C-1), 25.09 (C-2), 74.29 (C-3), 38.10 (C-4), 49.20 (C-5), 18.78 (C-6), 32.27 (C-7), 40.79 (C-8), 48.82 (C-9), 37.31 (C-10), 17.25 (C-11), 35.38 (C-12), 41.43 (C-13), 39.22 (C-14), 31.33 (C-15), 31.88 (C-16), 51.49 (C-17), 160.77 (C-18), 116.69 (C-19), 29.51 (C-20), 33.69 (C-21), 33.42 (C-22), 28.67 (C-23), 15.37 (C-24), 22.45 (C-25), 22.15 (C-26), 26.32 (C-27), 184.22 (C-28), 29.32 (C-29), 28.64 (C-30); ESI MS  $m/z$  (rel. int.): 456  $[\text{M}]^+$  ( $\text{C}_{30}\text{H}_{48}\text{O}_3$ ) (10.7), 440 (100), 316 (8.1), 304 (6.8), 152 (9.2), 140 (12.7).

#### 2 $\beta$ -Acetoxy-3-acetyl morolic acid (6)

Elution of the column with chloroform - methanol (19 : 1) produced colourless crystals of **6**, recrystallized from chloroform - methanol (1 : 1), 143 mg, m. p. 223 - 225°C; UV  $\lambda_{\text{max}}$  (MeOH): 215 nm; IR  $_{\text{max}}$  (KBr): 3231, 2948, 2871, 1733, 1694, 1645, 1455, 1366, 1249, 1036  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  5.17 (1H, s, H - 19), 4.39 (1H, brm,  $w_{1/2} = 14.5$  Hz, H-2 $\alpha$ ), 4.31 (1H, d,  $J = 8.5$  Hz, H - 3 $\alpha$ ), 2.11 (3H, br s, AcO), 2.02 (3H, br s, AcO), 1.07 (3H, br s, Me-23), 0.97 (3H, br s, Me-29), 0.93 (3H, br s, Me-25), 0.89 (3H, br s, Me-24), 0.87 (3H, brs, Me-26), 0.82 (3H, brs, Me-30), 0.78 (3H, br s, Me-27), 2.89 - 1.32 (23 H, m, 10 x  $\text{CH}_2$ , 3 x CH);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ ):  $\delta$  40.91 (C-1), 68.17 (C-2), 76.23 (C-3), 38.05 (C-4), 49.96 (C-5), 17.35 (C-6), 33.40 (C-7), 40.53 (C-8), 48.03 (C-9), 37.33 (C-10), 18.20 (C-11), 38.24 (C-12), 41.52 (C-13), 39.18 (C-14), 30.93 (C-15), 31.98 (C-16), 51.41 (C-17), 160.46 (C-18), 116.99 (C-19), 29.28 (C-20), 35.48 (C-21), 33.94 (C-22), 29.37 (C-23), 16.29 (C-24), 21.08 (C-25), 21.22 (C-26), 27.91 (C-27), 184.03 (C-28), 30.18 (C-29), 28.16 (C-30), 170.41, 2164, 22.42 (OAc); ESI MS  $m/z$  (rel. int.): 556  $[\text{M}]^+$  ( $\text{C}_{34}\text{H}_{52}\text{O}_6$ ) (9.1), 496 (31.2), 436 (10.1), 404 (6.2), 391 (25.4), 240 (8.8), 152 (4.8).

#### Betulinic acid 3 $\alpha$ -D-xylopyranoside (7)

Elution of the column with chloroform - methanol (9 : 1) offered colourless crystals of **7**, recrystallized from methanol, 168 mg, m. p. 287 - 299°C; UV  $\lambda_{\text{max}}$  (MeOH): 211 nm; IR  $_{\text{max}}$  (KBr): 3481, 3215, 2925, 2847, 1692, 1642, 1455, 1315, 1260, 1191, 1041, 883  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ ):  $\delta$  4.66 (1H, s,  $\text{H}_2$  - 29a), 4.53 (1H, s,  $\text{H}_2$  - 29b), 3.88 (1 H, dd,  $J = 5.5, 9.2$  Hz, H-3 $\alpha$ ), 1.61 (3H, br s, Me-30), 1.18 (3H, br s, Me-23), 0.90 (3H, br s, Me-25), 0.88 (3H, br s, Me-26), 0.74 (3H, brs, Me-24), 0.68 (3H, br s, Me-27), 2.91 - 1.34 (25 H, m, 10 x  $\text{CH}_2$ , 5 x CH), 5.47 (1H, d,  $J = 4.2$  Hz, H-1'), 4.06



(1H, dd, J = 4.2, 6.6 Hz, H-2'), 3.65 (1H, m, H-4'), 3.45 (2H, d, J = 7.8 Hz, H<sub>2</sub>-5'), 3.14 (1H, m, H-3'); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 41.32 (C-1), 29.28 (C-2), 74.27 (C-3), 38.93 (C-4), 56.27 (C-5), 18.61 (C-6), 36.25 (C-7), 40.31 (C-8), 47.12 (C-9), 36.75 (C-10), 22.16 (C-11), 25.51 (C-12), 40.16 (C-13), 42.13 (C-14), 31.81 (C-15), 26.25 (C-16), 55.03 (C-17), 48.98 (C-18), 41.46 (C-19), 150.31 (C-20), 30.88 (C-21), 33.91 (C-22), 29.09 (C-23), 15.51 (C-24), 16.27 (C-25), 17.28 (C-26), 23.36 (C-27), 178.45 (C-28), 110.17 (C-29), 23.32 (C-30), 103.21 (C-1'), 76.08 (C-2'), 72.15 (C-3'), 69.48 (C-4'), 62.07 (C-5'); ESI MS m/z (rel. int.): 588 [M]<sup>+</sup> (C<sub>35</sub>H<sub>56</sub>O<sub>7</sub>) (2.8), 455 (8.4), 411 (99.5), 393 (13.1).

### Isolation of phytoconstituents from *N. jatamansi*

#### 1-Oleo-2,3-distreoglyceride (8)

Elution of the column with petroleum ether yielded a pale yellow semisolid mass of **8**, yield 135 mg; UV λ max (MeOH): 215 nm; IR γ<sub>max</sub> (KBr): 2918, 2841, 1735, 1723, 1641, 1463, 1260, 725 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 5.31 (2H, m, H-9', H-10'), 4.15 (1H, m, H-2), 4.07 (2H, m, H<sub>2</sub>-1), 4.03 (2H, m, H<sub>2</sub>-3), 2.33 (2H, t, J = 7.2 Hz, H<sub>2</sub>-2'), 2.25 (4H, m, H<sub>2</sub>-2'', H<sub>2</sub>-2'''), 1.85 (2H, m, H<sub>2</sub>-8), 1.76 (2H, m, H<sub>2</sub>-11'), 1.53 (2H, m, CH<sub>2</sub>), 1.29 (56H, brs, 28 x CH<sub>2</sub>), 1.26 (12 H, brs, 6 x CH<sub>2</sub>), 1.20 (12H, brs, 6 x CH<sub>2</sub>), 0.89 (3H, t, J = 6.1 Hz, Me-18'), 0.86 (3H, t, J = 6.2 Hz, Me-18''), 0.82 (3H, t, J = 6.1 Hz, Me-18'''); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 173.25 (C-1'), 172.85 (C-1''), 169.83 (C-1'''), 131.09 (C-9'), 128.51 (C-10'), 80.04 (C-2), 64.41 (C-2), 63.24 (C-3), 40.48 - 29.66 (13 x CH<sub>2</sub>), 29.61 (24 x CH<sub>2</sub>), 29.48 - 25.27 (7 x CH<sub>2</sub>), 22.98 (CH<sub>2</sub>), 22.68 (CH<sub>2</sub>), 16.21 (Me-18'), 14.16 (Me-18''), 14.15 (Me-18'''); ESI MS m/z (ret. int.): 888 [M]<sup>+</sup> (C<sub>57</sub>H<sub>108</sub>O<sub>6</sub>) (2.1), 267 (33.8), 265 (22.9).

#### n-Nonyl stearate (9)

Elution of the column with petroleum ether - chloroform (3 : 1) gave colourless crystals of **9**, recrystallized from acetone - methanol (1:1), 205 mg, m. p. 86 - 87 °C, UV λ<sub>max</sub> (MeOH): 205 nm (log ε 4.5); IR γ<sub>max</sub> (KBr): 2922, 2849, 1733, 1645, 1456, 1378, 1252, 1175, 725 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 4.16 (2H, t, J = 7.6 Hz, H<sub>2</sub>-1'), 2.33 (2H, t, J = 7.2 Hz, H<sub>2</sub>-2), 2.06 (2H, m, CH<sub>2</sub>), 1.66 (2H, m, CH<sub>2</sub>), 1.55 (2H, m, CH<sub>2</sub>), 1.32 (4H, m, 2 x CH<sub>2</sub>), 1.29 (12H, brs, 6 x CH<sub>2</sub>), 1.25 (22H, brs, 11 x CH<sub>2</sub>), 0.89 (3H, t, J = 6.6 Hz, Me-9'), 0.85 (3H, t, J = 6.8 Hz, Me-18); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 173.11 (C-1), 61.17 (C-1'), 51.45 (C-2), 34.48 (CH<sub>2</sub>), 31.97 (CH<sub>2</sub>), 29.77 (CH<sub>2</sub>), 29.62 (12 x CH<sub>2</sub>), 29.47 (CH<sub>2</sub>), 29.39 (CH<sub>2</sub>), 29.28 (CH<sub>2</sub>), 29.13 (CH<sub>2</sub>), 26.96 (CH<sub>2</sub>), 24.96 (CH<sub>2</sub>), 22.68 (CH<sub>2</sub>), 14.29 (Me-9'), 14.18 (Me-18); ESI MS m/z (rel. int.): 410 [M]<sup>+</sup> (C<sub>27</sub>H<sub>54</sub>O<sub>2</sub>) (14.9), 283 (13.1), 267 (31.5).

#### Stearyl stearate (10)

Elution of the column with chloroform afforded colourless crystals of **10**, recrystallized from acetone - methanol (1:1); 121 mg; m. p. 98 - 100 °C; IR γ<sub>max</sub> (KBr): 2927, 2847, 1734, 1643, 1462, 1264, 1173, 1112, 727 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 4.05 (2H, t, J = 6.9 Hz, H<sub>2</sub>-1'), 2.33 (2H, t, J = 7.3 Hz, H<sub>2</sub>-2), 1.54 (2H, m, CH<sub>2</sub>), 1.23 (6H, brs, 3 x CH<sub>2</sub>), 1.21 (8H, brs, 4 x CH<sub>2</sub>), 1.19 (44H, brs, 22 x CH<sub>2</sub>), 0.86 (3H, t, J = 6.7 Hz, Me-18), 0.83 (3H, t, J = 6.3 Hz, Me-18'); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 171.23 (C-1), 65.29 (C-1'), 31.93 (CH<sub>2</sub>), 29.13 (27 x CH<sub>2</sub>), 29.41 (CH<sub>2</sub>), 29.27 (CH<sub>2</sub>), 29.19 (CH<sub>2</sub>), 22.73 (CH<sub>2</sub>), 14.13 (Me-18), 12.15 (Me-18'). +ve TOF MS m/z (rel.int.): 536 [M]<sup>+</sup> (C<sub>36</sub>H<sub>72</sub>O<sub>2</sub>) (28.3), 267 (13.1).

#### Lupenyl 3β-O-galactopyranosyl oleate (11)

Elution of the column with chloroform - methanol (19 : 1) furnished colorless crystals of **11**, recrystallized from chloroform - methanol (1 : 1), 133 mg, m. p. 221 - 224 °C, UV λ<sub>max</sub> (MeOH): 213 nm; IR γ<sub>max</sub> (KBr): 3512, 3328, 2928, 2841, 1742, 1645, 1463, 1375, 1178, 882, 723 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 4.81 (1H, br s, H<sub>2</sub>-29a), 4.68 (1H, br s, H<sub>2</sub>-29b), 4.03 (1H, dd, J = 5.3, 8.7 Hz, H-3α), 1.63 (3H, br s, Me-30), 1.08 (3H, br s, Me-23), 1.04 (3H, br s, Me-25), 0.93 (3H, br s, Me-27), 0.89 (3H, br s, Me-28), 0.86 (3H, br s, Me-26), 0.82 (3H, br s, Me-24), 5.12 (1H, d, J = 7.3 Hz, H-1'), 4.56 (1H, dd, J = 7.3, 6.6 Hz, H-2'), 4.11 (1H, m, H<sub>2</sub>-5'), 3.69 (1H, m, H-3'), 3.65 (1H, m, H-4'), 3.16 (2H, m, H<sub>2</sub>-6'), 5.33 (1H, m, H-9'), 5.31 (1H, m, H-10'), 2.32 (2H, t, J = 7.2 Hz, H<sub>2</sub>-2''), 2.25 (2H, m, H<sub>2</sub>-8''), 2.15 (2H, m, H<sub>2</sub>-11''), 0.80 (3H, t, J = 6.3 Hz, Me-18''), 1.25 (24H, brs, 12 x CH<sub>2</sub>), 2.23-1.29 (25 H, m, 10 x CH<sub>2</sub>, 5 x CH); <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 38.25 (C-1), 27.95 (C-2), 82.43 (C-3), 39.05 (C-4), 51.46 (C-5), 18.52 (C-6), 31.86 (C-7), 40.05 (C-8), 50.75 (C-9), 37.55 (C-10), 22.58 (C-11), 24.21 (C-12), 39.26 (C-13), 43.71 (C-14), 28.61 (C-15), 35.38 (C-16), 55.77 (C-17), 53.23 (C-18), 49.95 (C-19), 153.59 (C-20), 31.23 (C-21), 40.57 (C-22), 28.76 (C-23), 14.27 (C-24), 19.06 (C-25), 20.61 (C-26), 24.62 (C-27), 20.15 (C-28), 108.45 (C-29), 19.24 (C-30), 106.19 (C-1'), 85.83 (C-2'), 71.36 (C-3'), 64.37 (C-4'), 75.46 (C-5'), 60.19 (C-6'), 173.98 (C-1''), 35.38 (C-2''), 29.68 (C-3''), 29.63 (C-4''), 29.26 (C-5''), 29.12 (C-6''), 29.58 (C-7''), 34.86 (C-8''), 129.79 (C-9''), 129.98 (C-10''), 32.44 (C-11''), 29.48 (C-12''), 29.43 (C-13''), 29.32 (C-14''), 27.16 (C-15''), 26.87 (C-16''), 22.37 (C-17''), 14.08 (C-18''); ESI MS m/z (rel. int.): 852 [M]<sup>+</sup> (C<sub>54</sub>H<sub>92</sub>O<sub>7</sub>) (12.3), 425 (3.7), 281 (35.2), 265 (8.3).

## Results and Discussion

Compound **1**, named 3 $\beta$ -capryl 2 $\beta$ -hydroxybetulinic acid, showed distinctive IR absorption bands for hydroxyl group (3466 cm<sup>-1</sup>), ester function (1721 cm<sup>-1</sup>), carboxylic group (3281, 1686 cm<sup>-1</sup>), unsaturation (1635 cm<sup>-1</sup>) and long aliphatic chain (721 cm<sup>-1</sup>). Its molecular ion peak was determined on the basis of mass and <sup>13</sup>C NMR spectra at m/z 598 consistent with a molecular formula of a triterpenic ester, C<sub>38</sub>H<sub>62</sub>O<sub>5</sub>. The ion peaks arising at m/z 127 [C<sub>1</sub>' - O fission, CH<sub>3</sub>-(CH<sub>2</sub>)<sub>6</sub>-CO]<sup>+</sup>, 471 [M - 127, C<sub>30</sub>H<sub>47</sub>O<sub>4</sub>]<sup>+</sup> and 455 [M - CH<sub>3</sub>-(CH<sub>2</sub>)<sub>6</sub>-COO, C<sub>30</sub>H<sub>47</sub>O<sub>3</sub>]<sup>+</sup> indicated that caprylic acid was esterified with betulinic acid derivative. The <sup>1</sup>H NMR spectrum of **1** exhibited two one-proton singlets at  $\delta$  4.68 and 4.58 accounted to vinylic methylene H<sub>2</sub>-29 of a lupene-type triterpene, a one-proton doublet at  $\delta$  4.23 (J = 5.2 Hz) and a one-proton triple doublet at  $\delta$  3.70 (J = 4.4, 5.2, 8.8 Hz) ascribed to  $\alpha$ -oriented oxymethine H-3 and carbinol H-2 protons, respectively. A three-proton singlet in the deshielded region at  $\delta$  1.64 was attributed to C-30 methyl protons located on C-20 vinylic carbon. Six three-proton singlets between 1.23 - 0.65 were associated with the tertiary C-23 to C-28 methyl protons. A three-proton triplet at  $\delta$  0.84 with coupling interaction of 6.3 Hz was accommodated in the primary C-8' methyl protons. A two-proton triplet at  $\delta$  2.23 (J = 7.2 Hz) was due to methylene H<sub>2</sub>-2' protons adjacent to the ester group. The signals between  $\delta$  2.98 - 1.01 were due to the remaining methylene and methine protons. The <sup>13</sup>C NMR spectrum of **1** displayed signals for ester carbon at  $\delta$  174.41 (C-1'), vinylic carbons at  $\delta$  150.23 (C-20) and 109.58 (C-29), oxymethine carbons at  $\delta$  69.12 (C-2) and 76.73 (C-3), carboxylic carbon at  $\delta$  177.10 (C-28) and methyl carbons between  $\delta$  29.05 - 14.33. The <sup>1</sup>H and <sup>13</sup>C NMR spectral values of the triterpenic unit were compared with related lupene-type molecules [44,45]. On the basis of spectral data analysis and chemical reactions, the structure of **1** was formulated as lup-20(29)-en-2 $\beta$ -ol 3 $\beta$ -octanoyloxy-28-oic acid, a new lupene-type triterpenic ester.

Compound **2**, named 3 $\beta$ -caprioyl 2 $\beta$ -hydroxybetulinic acid, [M]<sup>+</sup> at m/z 626 (C<sub>40</sub>H<sub>66</sub>O<sub>5</sub>), was homologue of **1**. Its mass spectrum displayed ion fragments at m/z 155 [CH<sub>3</sub>-(CH<sub>2</sub>)<sub>8</sub>-CO]<sup>+</sup>, 171 [CH<sub>3</sub>-(CH<sub>2</sub>)<sub>8</sub>-COO]<sup>+</sup>, 471 [M - 155]<sup>+</sup> and 455 [M - 171]<sup>+</sup> suggesting that capric acid was esterified with the triterpenol. The <sup>1</sup>H NMR spectrum of **2** showed vinylic methylene H<sub>2</sub>-29 protons as one-proton singlets at  $\delta$  4.68 and 4.55,  $\alpha$ -oriented oxymethine H-3 proton as a one-proton doublet at  $\delta$  4.22 (J = 5.2 Hz), carbinol H-2  $\alpha$  proton as a one-proton triple doublet at  $\delta$  3.72 (J = 4.6, 5.2, 8.9 Hz), methyl protons as a three-proton singlet from  $\delta$  1.64 to 0.65 and as a three-proton triplet at  $\delta$  0.84 (J = 6.5 Hz, Me-10') and methine and methylene proton signals between  $\delta$  2.96 - 1.26. The <sup>13</sup>C NMR spectrum of **2** displayed signals for ester carbon at  $\delta$  174.26 (C-1'), vinylic carbons at  $\delta$  150.27 (C-20) and 109.61 (C-29), oxymethine carbons at  $\delta$  69.25 (C-2) and 76.74 (C-3), carboxylic carbon at  $\delta$  177.20 (C-

28) and methyl carbons between  $\delta$  28.53 - 14.31 [44,45]. On the basis of these evidences the structure of **2** was established as lup-20(29)-en-2 $\beta$ -ol, 3 $\beta$ -decanoyloxy-28-oic acid, a new lupene-type triterpenic ester.

Compound **3**, named 3 $\beta$ -oleiyl 2 $\beta$ -hydroxybetulinic acid, [M]<sup>+</sup> at m/z 736 (C<sub>48</sub>H<sub>80</sub>O<sub>5</sub>), was a homologue of **1** and showed mass ion fragments at m/z 265 [CH<sub>3</sub>-(CH<sub>2</sub>)<sub>7</sub>-CH = CH-(CH<sub>2</sub>)<sub>7</sub>CO]<sup>+</sup>, 281 [CH<sub>3</sub>-(CH<sub>2</sub>)<sub>7</sub>-CH = CH-(CH<sub>2</sub>)<sub>7</sub>COO]<sup>+</sup>, 471 [M - 265]<sup>+</sup> and 455 [M - 281]<sup>+</sup> suggesting that oleic acid was esterified with the triterpenol. The <sup>1</sup>H NMR spectrum of **3** showed two one-proton multiplets at  $\delta$  5.32 and 5.30 assigned to vinylic H - 9' and H - 10' protons, respectively, two one-proton singlets at  $\delta$  4.68 and 4.55 ascribed to unsaturated methylene H<sub>2</sub>-29 protons of a lupene-type triterpene, two one-proton signals as a doublet at  $\delta$  4.22 (J = 5.3 Hz) and as a triple doublets at  $\delta$  3.76 (J = 5.3, 5.5, 8.5 Hz) accounted correspondingly to  $\alpha$ -oriented oxymethine H-3 and H-2 protons, methyl protons between  $\delta$  1.68 - 0.65 and other methine and methylene protons from  $\delta$  2.96 to 1.30. The <sup>13</sup>C NMR spectrum of **3** displayed signals for ester carbon at  $\delta$  174.44 (C-1'), vinylic carbons at  $\delta$  150.27 (C-20), 109.61 (C-29), 129.66 (C-9') and 127.70 (C-10'), oxymethine carbons at  $\delta$  69.75 (C-2) and 76.73 (C-3), carboxylic carbon at  $\delta$  177.16 (C-28) and methyl carbons between  $\delta$  29.04 - 14.33 [44-46]. According to the spectral data analysis performed, compound **3** was characterized as lup-20(29)-en-2 $\beta$ -ol, 3 $\beta$ -octadec-9'-enoyloxy 28-oic acid, a new lupene-type triterpenic ester.

Compounds **4** and **5** were the known pentacyclic triterpenoids identified as betulinic acid and morolic acid (olean-18-en-3 $\beta$ -ol-28-oic acid), respectively [7,8].

Compound **6** responded positive tests for triterpenic acids and showed IR absorption bands for carboxylic function (3231, 1694 cm<sup>-1</sup>), ester groups (1748 cm<sup>-1</sup>) and unsaturation (1645 cm<sup>-1</sup>). Its mass spectrum displayed a molecular ion peak at m/z 556 consistent with a molecular formula of a diacetoxytriterpenic acid, C<sub>34</sub>H<sub>52</sub>O<sub>6</sub>. The ion peaks generated at m/z 496 [M - AcOH]<sup>+</sup>, 436 [496 - AcOH]<sup>+</sup> and 391 [436 - COOH]<sup>+</sup> suggested the existence of two acetoxy groups and one carboxylic function in the molecule. The ion fragments arising at m/z 240 [C<sub>5,6</sub> - C<sub>9,10</sub> fission]<sup>+</sup> and 152, 404 [C<sub>16,17</sub> - C<sub>13,18</sub> fission]<sup>+</sup> indicated the presence of the two acetoxy groups in ring A and vinylic linkage in ring E. The <sup>1</sup>H NMR spectrum **6** exhibited a one-proton singlet at  $\delta$  5.17 assigned to vinylic H-19 proton, a one-proton multiple at  $\delta$  4.39 with half-width of 14.5 Hz and a one-proton doublet at  $\delta$  4.31 (J = 8.5 Hz) ascribed to  $\alpha$ -oriented oxymethine H-2 and H-3 protons, respectively, two three-proton singlets at  $\delta$  2.11 and 2.02 due to acetoxy protons and seven three-proton singlets between  $\delta$  1.07 - 0.78 associated with the tertiary methyl protons of an oleanene-type

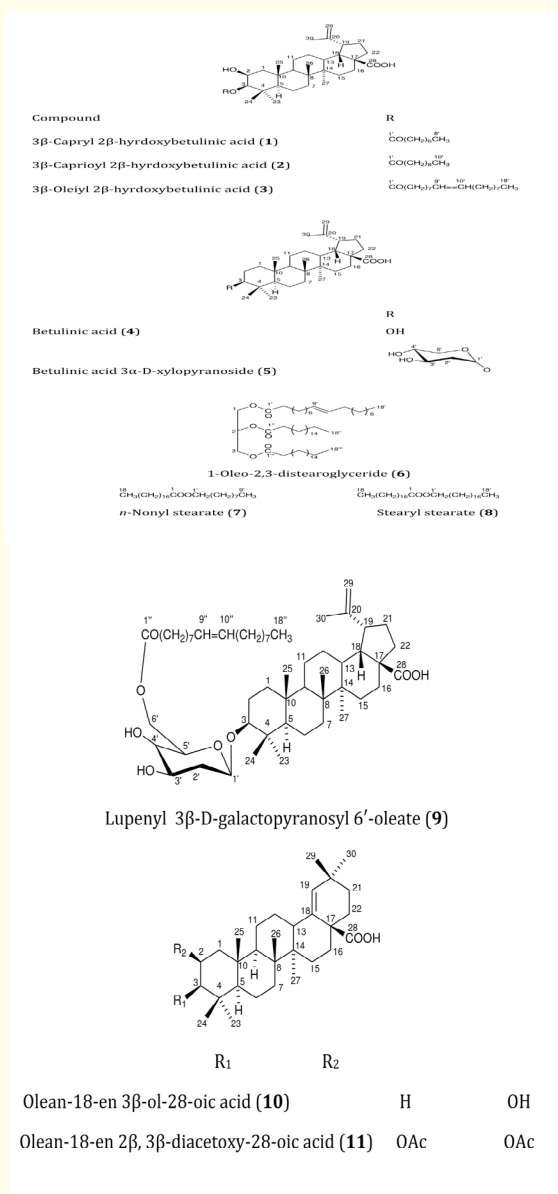
triterpenoid. The  $^{13}\text{C}$  NMR spectrum of **6** displayed signals for carboxylic carbon at  $\delta$  184.03 (C-28), vinylic carbons at  $\delta$  160.46 (C-18) and 116.99 (C-19), acetoxy carbons at  $\delta$  170.41, 21.64 and 22.42 and methyl carbons between  $\delta$  30.18 - 16.29 [45,46]. These evidences led to established structure of **6** as 2 $\beta$ -acetoxy-3-acetyl morolic acid, a new diacetoxy triterpenic acid.

Compound **7** gave positive tests for triterpenic glycoside and showed characteristic IR absorption bands for hydroxyl groups (3481, 3215  $\text{cm}^{-1}$ ), carboxylic function (1692  $\text{cm}^{-1}$ ) and unsaturation (1642  $\text{cm}^{-1}$ ). Its molecular ion peak was determined on the basis of mass and  $^{13}\text{C}$  NMR spectra at  $m/z$  588 consistent with a molecular formula of a triterpenic glycoside  $\text{C}_{35}\text{H}_{56}\text{O}_7$ . The ion peaks arising at  $m/z$  133 [ $\text{C}_1' - \text{O}$  fission,  $\text{C}_5\text{H}_9\text{O}_4$ ] $^+$  and 455 [ $\text{M} - 133$ ,  $\text{C}_{30}\text{H}_{47}\text{O}_3$ ] $^+$  indicated that a pentose unit was linked to a lupene-type triterpene.

The  $^1\text{H}$  NMR spectrum of **7** exhibited two one - proton singlets at  $\delta$  4.66 and 4.53 due to vinylic  $\text{H}_{2-29}$  methylene protons of the lupene-type triterpenic unit. A one - proton doublet at  $\delta$  4.03 ( $J = 5.5, 9.2$  Hz) was ascribed to oxymethine H-3 $\alpha$  proton. A one - proton doublet at  $\delta$  5.47 ( $J = 4.2$  Hz) was accounted to  $\beta$ -oriented anomeric H-1' proton. The other sugar protons appeared as a one-proton double doublet at  $\delta$  4.06 ( $J = 4.2, 6.6$  Hz) assigned to hydroxymethine H-2' proton, two one - proton multiplets at  $\delta$  3.65 (H-4') and 3.14 (H-3') and as a two - proton doublet at  $\delta$  3.45 ( $J = 7.8$  Hz) attributed to oxymethylene  $\text{H}_{2-5'}$  protons. A three - proton singlet in the deshielded region at  $\delta$  1.61 was accounted to C-30 methyl protons located on C-20 vinylic carbon. The other methyl signals appeared as three - proton singlets from  $\delta$  1.18 to 0.68. The  $^{13}\text{C}$  NMR spectrum of **7** displayed signals for vinylic carbons at  $\delta$  156.99 (C-20) and 109.15 (C-29), anomeric carbon at  $\delta$  103.21 (C-1'), oxymethine carbon at  $\delta$  74.27 (C-3), other sugar carbons between  $\delta$  76.08 - 62.07 and methyl carbons from  $\delta$  29.09 to 15.51. The carbon signals of the triterpenic unit were compared with the related lupene-type molecules [44,45]. Acid hydrolysis of **7** yielded betulinic acid, and  $\alpha$ -L xylose, co-TLC comparable. On the basis of these evidences, the structure of **7** had been formulated as lup-20(29)-en-3 $\beta$ -ol-28-oic acid 3-O- $\alpha$ -L-xylopyroside, a new lupene-type triterpenic xyloside.

Compound **8** was a fixed oil component characterized as 1-oleo-2,3-distreoglyceride. Compounds **9** and **10** were the fatty esters identified as *n*-nonyl stearate and stearyl stearate, respectively.

Compound **11**, named lupenyl 3 $\beta$ -O-galactopyranosyl oleate, gave positive tests for triterpenic glycoside and showed distinctive IR absorption bands for hydroxyl groups (3512, 3328  $\text{cm}^{-1}$ ), ester function (1742  $\text{cm}^{-1}$ ), unsaturation (1645  $\text{cm}^{-1}$ ) and long aliphatic chain (723  $\text{cm}^{-1}$ ). Its molecular ion peak was determined on the basis of mass and  $^{13}\text{C}$  NMR spectra at  $m/z$  852 consistent with a molecular formula of a triterpenic glycosidic ester  $\text{C}_{54}\text{H}_{92}\text{O}_7$ . The ion peaks arising at  $m/z$  265 [ $\text{C}_1'' - \text{O}$  fission,  $\text{CH}_3(\text{CH}_2)_7\text{-CH=CH-(CH}_2)_7\text{COO}$ ] $^+$ , 281 [ $\text{C}_1' - \text{O}$  fission,  $\text{CH}_3(\text{CH}_2)_7\text{-CH=CH-(CH}_2)_7\text{COO}$ ] $^+$  and 425 [ $\text{M} - \text{oleoglycoside}$ ,  $\text{C}_{30}\text{H}_{49}\text{O}$ ] $^+$  indicated that oleic acid was linked to a hexose unit attached to a lupene-type triterpene. The  $^1\text{H}$  NMR spectrum of **11** exhibited two one - proton doublets at  $\delta$  5.33 and 5.31 assigned to vinylic H-9'' and H-10'' of the fatty acid unit, respectively, two one-proton singlets at  $\delta$  4.81 and 4.68 accounted to vinylic methylene  $\text{H}_2-29$  of a lupene - type triterpene and a one - proton double doublet at  $\delta$  4.03 ( $J = 5.3, 8.7$  Hz) attributed to  $\alpha$ -oriented oxymethine H-3 proton. A three - proton singlet in the deshielded region at  $\delta$  1.63 was accounted to C-30 methyl protons located on C-20 vinylic carbon. Six three - proton singlets between 1.08 - 0.82 were associated with the tertiary C-23 to C-28. A one-proton doublet at  $\delta$  5.12 ( $J = 7.3$  Hz) was due to anomeric H-1' proton. The other sugar protons appeared as one-proton multiplet between  $\delta$  4.56 - 3.65 and as a two-proton multiplet at  $\delta$  3.16. A three-proton triplet at  $\delta$  0.80 with coupling interaction of 6.3 Hz was accommodated in the primary C-18'' methyl protons. A two-proton triplet at  $\delta$  2.32 ( $J = 7.2$  Hz) was due to methylene  $\text{H}_{2-2''}$  protons adjacent to the ester group, a broad singlet at  $\delta$  1.25 (24H) and signals as multiplets from  $\delta$  2.25 to 1.29 were associated with the remaining methylene and methine protons. The  $^{13}\text{C}$  NMR spectrum of **11** displayed signals for ester carbon at  $\delta$  173.98 (C-1''), vinylic carbons at  $\delta$  153.59 (C-20), 108.45 (C-29), 129.79 (C-9'') and 129.98 (C-10''), anomeric carbon at  $\delta$  106.19 (C-1'), oxymethine carbon at  $\delta$  82.43 (C-3) and other sugar carbons from  $\delta$  85.83 to 60.19. The presence of  $^1\text{H}$  NMR signal of the sugar H-2' signal at  $\delta$  4.56 in the deshielded region and C-2' carbon signal at  $\delta$  85.83 suggested attachment of the ester linkage at C-2' carbon. The carbon signals of the triterpenic unit were compared with related lupene-type molecules [44 - 46]. Acid hydrolysis of **11** yielded lup-20 (29)-en-3 $\beta$ -ol,  $\beta$ -D-galactose and oleic acid, co-TLC comparable. On the basis of spectral data analysis and chemical reactions, the structure of **11** was formulated as lup-20(29)-en-3 $\beta$ -olyl-3-O- $\beta$ -D-galactopyranosyl-2'-oleate. This is a new lupene-type triterpenic glycosidic ester (Figure 1).



**Figure 1:** Structural Formulae of the Chemical Constituents 1-11.

## Conclusion

The stem bark of *Albizia lebbeck* possessed three 3 $\beta$ -acyl 2 $\beta$ -hydroxybetulinic acids. Phytochemical investigation of the leaves of *Leptospermum scoparium* led to isolate betulinic acid, morolic acid, diacetoxymorolic acid and betulinic acid 3 $\alpha$ -D-xylopyranoside. The methanolic extract of the roots of *Nardostachys jatamansi* fur-

nished lupenyl 3 $\beta$ -O-galactopyranosyl oleate, 1-oleo-2,3-distearoglyceride, *n*-nonyl stearate and stearyl stearate. This work has enhanced understanding about the phytoconstituents of the plants. These compounds may be used as chromatographic markers for standardization of these herbal drugs.



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## Conflict of Interest

No financial interest or any conflict of interest exists.

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