# **Journal of Pharmaceutical and Biological Sciences**

ISSN: 2320-1924; CODEN: JPBSEV Published by Atom and Cell Publishers © All Rights Reserved Available online at: http://www.jpabs.org/ @rígínal Artícle



# Aliphatic alcohols, alkylated aromatic and triterpenic constituents from the aerial parts of *Rhus chinensis* Mill.

Mohammed Ali<sup>1</sup>\*, Shahnaz Sultana<sup>1,2</sup> and Showkat Rasool Mir<sup>1</sup>

 <sup>1</sup>Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110 062, India.
<sup>2</sup>College of Pharmacy, Jazan University, Jazan, Saudi Arabia

Received: 14-11-2016 / Revised Accepted: 27-12-2016 / Published: 01-01-2017

# ABSTRACT

*Rhus chinensis* Mill. (Anacardiaceae), occurs as a deciduous tree in India, China, Korea, Japan and south eastern Asia. Its leaf decoction is used to treat haemoptysis, inflammations, laryngitis, skin rashes, snake bite, stomach-ache and traumatic fractures. The fruits and seeds are taken to relieve coughs, diarrhea, dysentery, fevers, jaundice, malaria, rheumatism, stomachache, indigestion, intestinal worms, skin diseases, vomiting and as an antitoxin. The powdered aerial parts of *R. chinensis* (1 kg) were extracted with methanol in a Soxhlet apparatus. The extract was concentrated in vacuum to yield a brown semisolid mass (121 g). It was dissolved in minimum amount of methanol to adsorb on silica gel for column for preparation of a slurry. The slurry was dried in air and subjected to silica gel column loaded in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in order of increasing polarity to isolate 1-heneicosanol (1), *n*-octacosan-9 $\alpha$ -ol (2), benzyloxyhexadecane (3), 2-*n*-hexadecanyl benzoic acid (4),  $\alpha$ -amyrin (5), 2 $\beta$ ,3 $\beta$ ,22 $\beta$ -trihydroxylanostan-23 $\beta$ ,25-olide (rhuslanostanolide, 6), 12-dehydrobetulinic acid 3-O- $\beta$ -D- glucopyranoside (7) and oleanolic acid 3-O- $\beta$ -D-glucopyranoside (8). The structures of all the isolated phytoconstituents have been established on the basis of spectral data analysis and chemical reactions.

ð

Keywords: Rhus chinensis, aerial parts, chemical constituents, isolation, characterization.

# INTRODUCTION

Rhus chinensis Mill. syn. R. osbeckii Carriere, R. semialata Murray (Anacardiaceae), occurs as a deciduous tree in India, China, Korea, Japan and south eastern Asia. It is found in the outer Himalayan ranges at an altitude of 1,000-23,00 m, the hills of Assam, Khasia, Naga and Sikkim in India, upper Burma, China and Japan<sup>[1-4]</sup>. Its leaf decoction is taken to treat haemoptysis, inflammations, laryngitis, skin rashes, snake bite, stomach-ache and traumatic fractures. The fruits and seeds are used to relieve coughs, diarrhea, dysentery, fevers, jaundice, malaria, rheumatism, stomachache, indigestion, intestinal worms, skin diseases, vomiting and as an antitoxin<sup>[5-7]</sup>. R. chinensis compounds possess strong antiviral, antibacterial. anticancer, hepatoprotective, antidiarrheal and antioxidant activities<sup>[7]</sup>. The fruits contained  $\beta$ -sitosterol, morolic acid, 1-0heptatriacontanoyl glycerol,  $\alpha$ -monpalmitin,

palmitic, protocatechuic and gallic acids and methyl, ethyl and propyl gallates<sup>[8]</sup>. Hexadecanoic acid, phytol and heptacosane were present in leaf essential oils of different location. N-Tetradecane (12.8%) was detected in Khaili sample of China<sup>[9]</sup>. The leaves possessed quercetin, its 3-rhamnoside, hyperoside, quercetin and kaempferol<sup>[10,11]</sup>. This paper describes isolation and characterization of chemical constituents from the aerial parts of *R. chinensis*.

# MATERIALS AND METHODS

**General procedure:** Melting points were determined on a Perfit apparatus without correction. The infrared (IR) spectra were measured in KBr pellet on a Bio-Rad Fourier transform-IR spectrometer (Spectra Lab Scientific Inc., Ontario, Canada). Ultraviolet (UV) spectra were obtained in methanol with a Lambda Bio 20 spectrometer (Perkin-Elmer, Rotkreuz,

\*Corresponding Author Address: Dr. Mohammed Ali, Phytochemistry Research Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110 062, India; E-mail: maliphyto@gmail.com

Switzerland). <sup>1</sup>H (400 MHz) and <sup>13</sup>C (100 MHz) nuclear magnetic resonance (NMR) spectra were recorded on Bruker spectrospin spectrometer (Bruker AXS GmbH, Karlsruhe, Germany), CDCl<sub>3</sub> (Sigma-Aldrich, Bengaluru, India) was used as a solvent and TMS as an internal standard. Mass spectra were scanned by effecting ionization at 70 eV on a JEOL-JMS-DX 303 spectrometer (Japan) equipped with direct inlet probe system. Column chromatography separations were carried out on silica gel (Merck, 60-120 mesh, Mumbai, India). Precoated silica gel plates (Merck, Silica gel 60 F254) were used for analytical thin layer chromatography and visualized by exposure to iodine vapors and UV radiations.

**Plant material**: The aerial parts of *R. chinensis* were collected from eastern Sikkim and identified by Dr. H. B. Singh, In-charge, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi.

**Extraction and isolation:** The air dried coarsely powdered aerial parts of *R. chinensis* (1 kg) were extracted with methanol using a Soxhlet apparatus for 18 h. The extract was concentrated in vacuum to yield a brown semisolid mass (121 g). A small portion of the extract was analyzed chemically to determine the presence of different chemical constituents. The extract (100 g) was dissolved in minimum amount of methanol to adsorb on silica gel (60–120 mesh) for preparation of a slurry. The slurry was dried in air and subjected to silica gel column loaded in petroleum ether. The column was eluted with petroleum ether, chloroform and methanol in order of increasing polarity to isolate the following compounds:

**1-Heneicosanol (1):** Elution of the column with petroleum ether – chloroform (1:1) gave white shiny flakes of **1**, 97 mg, m. p. 70-71 °C; IR γmax (KBr): 3403, 2915, 2846, 1463, 1381, 1121, 1024, 722 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): $\delta$  3.39 (2H, t, *J* = 6.8 Hz, CH<sub>2</sub>-1), 1.63 (2H, m, H<sub>2</sub>-2), 1.49 (2H, m, CH<sub>2</sub>), 1.25 (34H, brs, 17 × CH<sub>2</sub>), 0.81 (3H, t, *J* = 6.5 Hz, Me-21); <sup>13</sup>C NMR (CDCl<sub>3</sub>): $\delta$  63.21 (C-1), 32.93-22.69 (19 × CH<sub>2</sub>), 14.13 (Me-25); EIMS *m/z* (rel. int.): 312 [M]<sup>+</sup> (C<sub>21</sub>H<sub>44</sub>O) (15.2).

*n*-Octacosan-9*a*-ol (2): Elution of the column with petroleum ether – chloroform (1:3) afforded colourless crystals of 2, 115 mg, m. p. 87-89° C; IR  $\gamma$ max (KBr): 3407, 2914, 2852, 1457, 1382, 1158, 1073, 725 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  4.07 (1H, m, w<sub>1/2</sub> = 5.1 Hz, H-9), 1.53 (2H, m, H<sub>2</sub>-8), 1.50 (2H, m, H<sub>2</sub>-10), 1.23 (14H, brs, 7 × CH<sub>2</sub>), 1.20 (32H, brs, 17 x CH<sub>2</sub>), 0.85 (3H, t, *J* = 6.7 Hz, Me-1), 0.82 (3H, t, *J* = 6.1 Hz, Me-28); <sup>13</sup>C NMR

(CDCl<sub>3</sub>):  $\delta$  68.32 (C-9), 34.22 (C-8), 32.93 (C-10), 30.45 (CH<sub>2</sub>), 34.22 (C-8), 32.93 (C-10), 30.45 (CH<sub>2</sub>), 29.33 (3 × CH<sub>2</sub>), 29.25 (13 x CH<sub>2</sub>), 27.81 (CH<sub>2</sub>), 24.85 (CH<sub>2</sub>), 22.68 (CH<sub>2</sub>), 14.16 (Me-1), 14.07 (Me-28); EIMS *m*/*z* (rel. int.): 410 [M]<sup>+</sup> (C<sub>28</sub>H<sub>58</sub>O) (6.4), 392 (100), 297 (13.6), 267 (8.1), 143 (41.2), 85 (23.5), 57 (65.9).

**Benzyloxyhexadecane** (**3**): Elution of the column with chloroform yielded pale yellow crystals of **3**, 151 mg; m.p. 80-82 °C; UV  $\lambda$ max (MeOH): 225, 280 nm (log  $\varepsilon$  4.1, 3.7); IR  $_{1}$ max (KBr): 2923, 2842, 1632, 1521, 1468, 1317, 1250, 1155, 1055, 890, 815. 728 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.28 (1H, m, H-3), 7.23 (1H, m, H-5), 6.84 (1H, m, H-2), 6.81 (1H, m, H-6), 6.78 (1H, m, H-4), 4.55 (2H, s, CH<sub>2</sub>-7), 3.09 (2H, t, J = 8.1 Hz, H<sub>2</sub>-1'), 1.63 (2H, m, H<sub>2</sub>-2'), 1.29 (12H, s, 6 x CH<sub>2</sub>), 1.25 (14H, s, 7 x CH<sub>2</sub>), 0.85 (3H, t, J = 6.8 Hz, Me-16'); EIMS *m*/*z* (rel. int.): 332 [M]<sup>+</sup> (C<sub>23</sub>H<sub>40</sub>O) (23.1), 107 (66.5), 225 (24.8).

2-Hexadecanyl benzoic acid (4): Elution of the column with chloroform - methanol (49 : 1) furnished colourless crystals of 4, 105 mg; m. p. 82-84 °C; UV λmax (MeOH): 225, 278 nm (log ε 4.1, 2.9); IR γmax (KBr): 3211, 2927, 2853, 1687, 1622, 1521, 1473, 1314, 1252, 1165, 1055, 897, 725 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  7.37 (1H, dd, J = 2.5, 8.3 Hz, H-6), 7.33 (1H, dd, J = 2.8, 7.9 Hz, H-3), 6.88 (1H, m, H-5), 6.76 (1H, m, H-4), 2.91  $(2H, t, J = 7.6 Hz, H_2-1'), 1.62 (2H, m, H_2-2'),$ 1.54 (2H, m, CH<sub>2</sub>), 1.34 (2H, m, CH<sub>2</sub>), 1.27 (10H, s, 5 x CH<sub>2</sub>), 1.25 (12H, s, 6 x CH<sub>2</sub>), 0.86 (3H, t, J = 6.8 Hz, Me-16'); EIMS m/z (rel. int.): 346  $[M]^+(C_{23}H_{38}O_2)$  (17.9), 301 (8.8), 225 (215), 121 (24.1).

**α-Amyrin (5):** Further elution of the column with chloroform – methanol (49 : 1) provided colourless powder of **5**, 217 mg, m. p. 185-187  $^{0}$ C, UV  $\lambda_{max}$  (MeOH): 205 nm (log ε 4.1); IR γmax (KBr): 3424, 2927, 2846, 1635, 1458, 1383, 1256, 1021, 965 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ δ 5.36 (1H, m, H-12), 3.31 (1H, dd, J=5.2, 9.3 Hz, H-3β), 2.18 (1H, d, J = 5.08 Hz, H-18β), 1.26 (3H, brs, Me-23), 1.15 (3H, brs, Me-28), 1.09 (3H, d, J = 6.3 Hz, Me-29), 1.02 (3H, brs, Me-24), 0.97 (3H, brs, Me-25), 0.89 (3H, d, J = 6.5 Hz, Me-30), 0.85 (3H, brs, Me-27), 0.69 (3H, brs, Me-26), 2.14 – 1.29 (23H, m, 9 x CH<sub>2</sub>; 5 x CH); EIMS *m*/*z* (rel. int.): 426 [M]<sup>+</sup> (C<sub>30</sub>H<sub>50</sub>O) (10.4).

**Rhuslanostanolide** (6): Elution of the column with chloroform-methanol (19:1) produced colourless crystals of 6, 63 mg m.p. 192-193°C; UV λmax (MeOH): 214 nm; IR γmax (KBr): 3417, 3397, 2917, 2848, 1734, 1638, 1464, 1376, 1263, 1176, 1127, 1061, 970, 909 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ

4.25 (1H, brm,  $w_{1/2} = 15.3$  Hz, H-23 $\alpha$ ), 3.76 (1H, dd, J = 4.9, 5.2 Hz, H-22 $\alpha$ ), 3.37 (1H, d, J = 5.2 Hz, H-3 $\alpha$ ), 3.18 (1H, ddd, J = 9.8, 5.2, 5.8 Hz, H-2 $\alpha$ ), 2.23 (1H, m, H-25), 2.18 (1H, m, H-9 $\alpha$ ), 2.15 (1H, m, H-5 $\alpha$ ), 1.58 (1H, m, H-17), 1.55 (1H, m, H-20), 1.15 (3H, brs, Me-30), 1.06 (6H, brs, Me-19, Me-29), 0.97 (3H, d, J = 6.3 Hz, Me-21), 0.90 (3H, s, Me-28), 0.86 (3H, d, J = 6.1 Hz, Me-26), 0.83 (3H, brs, Me-18), 2.35-1.33 (16 H, m, 8 × CH<sub>2</sub>); EIMS *m*/*z* (rel. int.): 490 [M]<sup>+</sup> (C<sub>30</sub>H<sub>50</sub>O<sub>5</sub>) (2.3), 333 (13.6), 157 (10.1).

12-Dehydrobetulinic acid 3-O- glucoside (7): Elution of the column with chloroform-methanol (9:1) offered colourless crystals of 7, 112 mg, m.p. 206-208°C; UV λmax (MeOH): 211 nm; IR γmax (KBr): 3441, 3395, 3271, 2928, 2852, 1697, 1642, 1472, 1373, 1251, 1186, 1162, 1018, 883 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 5.35 (1H, m, H-12), 5.09 (1H, d, J = 7.2 Hz, H-1'), 4.71 (1H, s, H<sub>2</sub>-29a), 4.66 (1H, s, H<sub>2</sub>-29b), 4.31 (1H, m, H-5'), 4.12 (1H, m, H-2'), 3.86 (1H, m, H-3'), 3.73 (1H, m, H-4'), 3.37 (1H, dd, J = 5.2, 9.1 Hz, H-3 $\alpha$ ), 3.04 (2H, d, J = 6.6 Hz, H<sub>2</sub>-6), 1.67 (3H, brs, Me-30), 1.37 (3H, brs, Me-23), 1.03 (3H, brs, Me-26), 0.97 (3H, s, Me-24), 0.93 (3H, brs, Me-25), 0.86 (3H, brs, Me-27), 2.27-1.39 (22 H, m,  $9 \times CH_2$ , 4 x CH); EIMS m/z (rel. int.): 616 [M]<sup>+</sup> (C<sub>36</sub>H<sub>56</sub>O<sub>8</sub>) (12.5).

**Oleanolic acid 3-O-glucoside (8)**: Further elution of the column with chloroform-methanol (9:1) afforded colourless crystals of **8**, 131 mg, m.p. 217-219°C; UV λmax (MeOH): 213 nm; IR γmax (KBr): 3415, 3385, 3266, 2927, 2842, 1703, 1645, 1473, 1365, 1253, 1190, 1028, 878 cm<sup>-1</sup>; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 5.33 (1H, m, H-12), 5.11 (1H, d, J = 7.3 Hz, H-1'), 4.69 (1H, m, H-5'), 4.55 (1H, m, H-2'), 4.24 (1H, m, H-3'), 3.71 (1H, m, H-4'), 3.34 (1H, dd, *J* = 5.3, 9.5 Hz, H-3α), 3.04 (2H, d, J = 6.6 Hz, H<sub>2</sub>-6), 1.31 (3H, brs, Me-23), 1.05 (3H, brs, Me-25), 0.98 (3H, brs, Me-30), 0.83 (3H, brs, Me-27), 2.27-1.39 (22 H, m, 9 × CH<sub>2</sub>, 4 x CH); EIMS *m*/*z* (rel.int.): 618 [M]<sup>+</sup> (C<sub>36</sub>H<sub>58</sub>O<sub>8</sub>) (8.3).

### **RESULTS AND DISCUSSION**

Compound 1,  $[M]^+$  at m/z 312 (C<sub>21</sub>H<sub>44</sub>O), showed IR absorption bands for hydroxyl group (3403 cm<sup>-1</sup>) and long aliphatic chain (722 cm<sup>-1</sup>). Its <sup>1</sup>H NMR spectrum exhibited a two-proton triplet at  $\delta$  3.39 (J = 6.8 Hz) assigned to hydroxymethylene H<sub>2</sub>-1 protons, other methylene protons as two-proton multiplets at  $\delta$  1.63 and 1.49 and as a singlet at  $\delta$  1.25 (34 H) and as a three-proton triplet at  $\delta$  0.81 (J = 6.5 Hz) accounted to terminal C-21 primary methyl protons. The <sup>13</sup>C NMR spectrum of 1 displayed signals for hydroxymethylene carbon at  $\delta$  63.21 (C-1), methylene carbons from  $\delta$  32.93 to

22.69 and methyl carbon at  $\delta$  14.13 (C-31). These evidences led to characterize the structure of **1** as 1-heneicosanol. It was detected in the leaf volatile oil of *Uncaria sessilifructus*<sup>[12]</sup> and in the bark of *Commiphora caudata*<sup>[13]</sup>.

Compound 2 had IR absorption bands for hydroxyl group (3407 cm<sup>-1</sup>) and long aliphatic chain (725 cm<sup>-1</sup>). Its mass spectrum exhibited a molecular ion peak at m/z 410 corresponding to an aliphatic alcohol C<sub>28</sub>H<sub>58</sub>O. The prominent ion peaks arising at m/z 392  $[M - H_2O]^+$ , 297  $[C_8 - C_9$  fission, CH<sub>3</sub>-(CH<sub>2</sub>)<sub>7</sub>]<sup>+</sup>, 143 [C<sub>9</sub> - C<sub>10</sub> fission, CH<sub>3</sub>-(CH<sub>2</sub>)<sub>7</sub>-CHOH]<sup>+</sup> and 267 [(CH<sub>2</sub>)<sub>18</sub>-CH<sub>3</sub>]<sup>+</sup> indicated the existence of the hydroxyl group at C<sub>9</sub> carbon. Its <sup>1</sup>H NMR spectrum showed a one-proton multiplet at  $\delta$  4.07 with half-width of 5.1 Hz assigned to  $\beta$ oriented carbinol H-9 proton. The methylene protons appeared as two-proton multiplets at  $\delta$  1.53 and 1.50 and as broad signals at  $\delta$  1.23 (14 H) and 1.20 (32 H). Two three-proton triplets at  $\delta$  0.85 (J = 6.5 Hz) and 0.82 (J = 6.1 Hz) were accounted to terminal C-1 and C-28 primary methyl protons, respectively. The <sup>13</sup>C NMR spectrum of 2 displayed signals for carbinol carbon  $\delta$  68.32 (C-9), methylene carbons from  $\delta$  34.22 to 22.68 and methyl carbons at  $\delta$  14.16 (C-1) and 14.07 (C-28). The absence of any signal beyond  $\delta$  4.07 in the <sup>1</sup>H NMR spectrum and  $\delta$  68.32 in the <sup>13</sup>C NMR spectrum supported saturated nature of the molecule. On the basis of above discussion structure of **2** was established as *n*-octacosan- $9\alpha$ -ol.

Compound 3 showed IR absorption bands for aromatic ring (1632, 1521, 1055 cm<sup>-1</sup>) and long aliphatic chain (815, 728 cm<sup>-1</sup>). Its mass spectrum exhibited a molecular ion peak at m/z 332 corresponding to a molecular formula of a benzyl ether  $C_{23}H_{40}O$ . The ion peaks arising at m/z 107 [O  $- C_{1'}$  fission,  $C_6 H_5$ -CH<sub>2</sub>-O]<sup>+</sup> and 225 [M - 107,  $(CH_2)_{15}CH_3]^+$ suggested the linkage of an hexadecanyl chain to the benzyloxy group. The <sup>1</sup>H NMR spectrum of **3** exhibited five one-proton multiplets between  $\delta$  7.28 - 6.78 assigned to aromatic protons. A two-proton signal  $\delta$  4.55 and a two-proton triplet at  $\delta$  3.09 (J = 8.1 Hz) were ascribed to oxymethylene H<sub>2</sub>-7 and H<sub>2</sub>-1' protons, respectively. The other methylene protons resonated as a two-proton multiplet at  $\delta$  1.63 (H<sub>2</sub>-2') and as singlets at  $\delta$  1.29 (6 x CH<sub>2</sub>) and 1.25 (7 x CH<sub>2</sub>). A three-proton triplet at  $\delta$  0.85 (J = 6.8 Hz) was ascribed to primary C-18 methyl protons. On the basis of these evidences the structure of 3 has been determined as benzyloxyhexadecane, a new aromatic ether.

Compound **4** yielded effervescences with sodium bicarbonate solution and showed IR absorption bands for carboxylic group (3211, 1687 cm<sup>-1</sup>),

aromatic ring (1622, 1521, 1055  $\text{cm}^{-1}$ ) and long aliphatic chain (725 cm<sup>-1</sup>). Its mass spectrum exhibited a molecular ion peak at m/z 346 corresponding to an alkyl substituted benzoic acid,  $C_{23}H_{38}O_2$ . The ion peaks generating at m/z 301 [M - COOH]<sup>+</sup>, 225 [C<sub>2</sub> - C<sub>1'</sub> fission, CH<sub>2</sub>(CH<sub>2</sub>)<sub>14</sub>CH<sub>3</sub>]<sup>+</sup> and 121  $[M - 225]^+$  supported the attachment of an *n*-hexadecanyl group to benzoic acid. The  ${}^{1}\text{H}$ NMR spectrum of 4 displayed two one-proton double doublets at  $\delta$  7.37 (J = 2.5, 8.3 Hz) and 7.33 (J = 2.8, 7.9 Hz) assigned to ortho-, meta- coupled aromatic H-6 and H-3 protons, respectively. Two one – proton multiplets at  $\delta$  6.88 and 6.76 were ascribed to aromatic H-5 and H-4 protons. respectively. A two-proton triplet at  $\delta 2.91$  (J = 7.6 Hz) was attributed to methylene H<sub>2</sub>-1' protons linked to the aromatic ring. The other methylene protons resonated as two-proton multiplets at  $\delta$ 1.62  $(H_2-2')$ , 1.54  $(CH_2)$  and 1.34  $(CH_2)$  and as singlets at  $\delta$  1.27 (5 x CH<sub>2</sub>) and 1.25 (6 x CH<sub>2</sub>). A three-proton triplet at  $\delta$  0.86 (J = 6.8 Hz) was accounted to primary C-16' methyl protons. On the basis of these evidences the structure of 4 has been elucidated as 2-n-hexadecanyl benzoic acid, a new alkylated aromatic acid.

Compound **5**,  $[M]^+$  at m/z 426 (C<sub>30</sub>H<sub>50</sub>O) was characterized as  $\alpha$ -amyrin on the basis of spectral data analysis and comparison of the physical parameters with the reported data<sup>[14]</sup>.

Compound 6, named rhuslanostanolide, responded positively to Liebermann-Burchardt test for triterpenoids and showed IR absorption bands for lactone ring (1734 cm<sup>-1</sup>) and hydroxyl groups (3417, 3397 cm<sup>-1</sup>). Its mass spectrum exhibited a molecular ion peak at m/z 490 corresponding to a molecular formula of a lanostanyl lactone,  $C_{30}H_{50}O_5$ . The ion fragments arising at m/z 157  $[C_{17} - C_{20}$  fission,  $C_8 H_{13} O_3]^+$  and 333 [M - 157,  $C_{22}H_{37}O_2$ <sup>+</sup> indicated the attachment of a saturated side chain with one hydroxyl group and lactone ring to the tetracyclic lanostene-type framework with two hydroxyl functions. The <sup>1</sup>H NMR spectrum of **6** displayed a one-proton multiplet at  $\delta$ 4.25 with half-width of 15.3 Hz assigned to oxymethine H-23a proton and three signals as a double doublet at  $\delta$  3.76 (J = 4.9, 5.2 Hz), as a doublet at  $\delta$  3,37 (J = 5.2 Hz) and as a triple doublet at  $\delta$  3.18 (J = 9.8, 5.2, 5.8 Hz) accounted

correspondingly to carbinol H-22 $\alpha$ . H-3 $\alpha$  and H-2 $\alpha$ protons. Two three-proton doublets at  $\delta$  0.97 (J = 6.3 Hz) and 0.86 (J = 6.1 Hz) and four singlets at  $\delta$ 1.15 (3H), 1.06 (6H), 0.90 (3H) and 0.83 (3H) were associated with the secondary C-21 and C-26 and tertiary C-30, C-19, C-29, C-28 and C-18 methyl protons, respectively, all of them were attached to saturated carbons. The other methine and methylene protons appeared between  $\delta 2.35 - 1.33$ . On the basis of above discussion the structure of 6has been elucidated as 2β,3β,22βtrihydroxylanostan-236,25-olide, a new lanostane type-triterpenic lactone.

Compound 7 responded positive tests for triterpenic glycosides and showed IR absorption bands for hydroxyl groups (3441, 3395 cm<sup>-1</sup>), carboxylic function (3271, 1697 cm<sup>-1</sup>) and unsaturation (1642 cm<sup>-1</sup>). Its mass spectrum displayed a molecular ion peak at m/z 616 consistent with a molecular formula of a triterpenic acid glycoside, C<sub>36</sub>H<sub>56</sub>O<sub>8</sub>. The <sup>1</sup>H NMR spectrum 7 exhibited signals attributable to an exomethylene protons at  $\delta$  4.71 and 4.66 (1H each, brs) together with an allylic methyl at  $\delta$  1.67 indicating an isopropenyl functionality. A one-proton multiplet at  $\delta$  5.35 and a one-proton doublet at  $\delta$  5.09 (J = 7.2 Hz) were ascribed to vinylic H-12 and anomeric H-1' protons, respectively. The other sugar protons resonated as one-proton multiplets between  $\delta$  4.31 -3.73 and as a two-proton doublet at  $\delta$  3.04 (J = 6.6 Hz, H<sub>2</sub>-6). A one-proton double doublet at  $\delta$  3.37 (J = 5.2, 9.1 Hz) was accounted to oxymethine H-3 $\alpha$ proton. Five three-proton signals from  $\delta$  1.37 to 0.86 were associated with tertiary C-23 to C-30 methyl protons. Acid hydrolysis of 7 yielded Dglucose, co-TLC comparable. These evidences led to established structure of 7 as 12-dehydrobetulinic acid 3-O-β-D- glucopyranoside.

Compound **8**,  $[M]^+$  at m/z 618 (C<sub>36</sub>H<sub>58</sub>O<sub>8</sub>), showed IR absorption bands for hydroxyl groups (3415, 3385 cm<sup>-1</sup>), carboxylic group (3266, 1703 cm<sup>-1</sup>) and unsaturation (1645 cm<sup>-1</sup>). It <sup>1</sup>H NMR spectrum exhibited signals for a vinylic proton ( $\delta$  5.33, m, H-12), anomeric proton ( $\delta$  5.11, d, J = 7.3 Hz, H-1'), other sugar and H-3 oxymethine protons ( $\delta$  4.69 – 3.04) and seven tertiary methyl protons ( $\delta$  1.31-0.83). The compound **8** was characterized as oleanolic acid 3-O- $\beta$ -D-glucopyranoside<sup>[15,16]</sup>.



1. Heneicosanol



**2**. *n*-Octacosanol-9α-ol



3. Benzyloxy hexadecane





4. 2-hexadecanyl benzoic acid

5. α-Amyrin





Phytochemical investigation of a methanolic extract of the aerial parts of *Rhus chinensis* resulted in the isolation of two aliphatic alcohols, a benzyloxyhexadecane, a 2-*n*-hexadecanyl benzoic acid and four triterpenoids. This work has enhanced understanding about the phytoconstituents of the plant. These compounds may be used as chromatographic markers for standardization of the aerial parts.

#### Acknowledgements

The authors are thankful to the instrumentation centers, Central Drug Research Institute, Lucknow and Jawaharlal Nehru University, New Delhi for recording spectral data of the compounds.

#### **Conflicts of interests**

We declare that we have no conflict of interest.

#### Ali et al., J Pharm Biol Sci 2017; 5(1): 1-7

#### REFERENCES

- 1. Bhattacharjee S.K. In: Handbook of Medicinal Plants. Bhattacharjee S K, editor. Jaipur, India: Pointer Publishers; 1998. p 299.
- 2. Rai L, Sharma E. Medical plants of the Sikkim Himalayan. Kalimpong, India, *Rhus semialata* Murr, Bishen Singh Mahendra publication; 1994. p 68.
- 3. Gurung G. *Rhus semialata* Murr. In: Gurung B, editor. The Medicinal Plants of Sikkim Himalaya. West Sikkim: Published by Jasmin Bijoy Gurung; 2002. p 339.
- 4. Kiritikar KR, Basu BD. *Rhus semialata* Murr. In: Blatter E, editor. Indian Medicinal Plants. Dehra Dun, India: International Book Distributors; 1987. pp 646–647.
- 5. Anonymous, author. *Rhus semialata* Murr. In: Krisnamurthi A, editor. The Wealth of India. Vol-IX. New Delhi, India: National Institute of Science Communication, CSIR, 2003. p 19.
- 6. Quattrocchi U. CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific names, Eponyms. Synonyms, and Etymology. CRC Press, Boca Raton, Florida, 2016. p 3210.
- 7. Djakpo O, Yao W, *Rhus chinensis* and *Galla chinensis*--folklore to modern evidence: review. Phytother. Research. 2010; 24(12): 1739-47.
- 8. Li B, Gao JY, Gong LM, Liu PA, Li SX. . Chemical Constituents from *Rhus chinensis* Fruit Dregs. Zhong Yao Cai. 2015; 38 (6): 1209-11.
- Zhu B, Ren Z, Nan P, Jiang M, Zhao J, Zhong Y. Chemical variation in leaf essential oils of *Rhus chinensis* from eight locations in Southern and Eastern China. Chem. Natural Compounds. 2007; 43 (6): 741–43.
- Qiu Z, Tang M, Deng G, Yang H, Zhang X, Huang S, Wu L. Antioxidant and antigenotoxic activities of ethanol extracts from *Rhus chinensis* Mill leaves. Food Science and Biotechnology. 2014; 23(4): 1213–21.
- 11. Tan D, Yan Q, Ma X. Chemical constituents of *Rhus chinensis*. Chem. Natural Compounds. 2015; 51(3): DOI: 10.1007/s10600-015-1336-2.
- 12. Dong-Mei W., Lin-Fang H.. Composition of volatile oil from the leaves of *Uncaria sessilifructus* Roxb. J. Applied Pharma. Sci. 2012; 2(11): 50-53.
- 13. Reddy S., Ammani Ch., Rose Mary K., Rajesh T N, Aravind D. G., Sekaran BC. Phytochemical and GC-MS analysis of *Commiphora caudata* (Wt. & Arn.) Eng. Bark. Indian J. Adv. Plant Research. 2014; 1 (5): 24 -29.
- 14. Chaudhary N, Husain SS, Ali M. Phytochemical investigation of the stem bark of *Ficus hispida* L. J. Sci. Innov. Research. 2014; 3(4): 1-5.
- 15. Ghosh D, Thejomoorthy P, Veluchamy. Anti-inflammatory and analgesic activities of oleanolic acid 3-/3- glucoside (RDG-1) from *Randia dumetorum* (Rubiaceae). Indian J. Pharmacology. 1983; 15(4): 331-42.
- 16. Szyja W, Wikomirski B, Kasprzyk Z. Biosynthesis of oleanolic acid glycosides in isolated ligulate flowers of of *Calendula officinalis*. Phytochemistry. 1983; 22(1): 111-13.