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# Stigmasterol, rengyolone, 2-phenylethyl β-D-glucopyranoside and n-tetradecyl-β-D-glucopyranoside from the flowers of *Nyctanthes arbor-tristis* Linn

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## Abstract

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# Introduction

Nyctanthes arbor-tristis Linn (Bengali name: Sheuliphul, Shefaliphul) is an important medicinal plant grown throughout the country (Ghani, 2003). This plant normally grows in tropical and sub tropical region (Rani et al., 2012). It is well known in Bangladesh and neighboring countries. It belongs to genus Nyctanthes and the family Oleaceae (Rathod et al., 2010). It was previously reported that the whole Nyctanthes arbor-tristis Linn plant is used for treatment of cancer (Kirtikar and Basu, 2002) while the leaf juice is used to expel roundworms and threadworms in children (Chauhan, 1999) in the treatment of loss of appetite, piles, liver disorders, biliary disorders, chronic fever, malarial fever, obstinate sciatica, rheumatism as well as a diaphoretic agent (Banerjee et al., 2007). The stem bark is used for the treatment of malaria and rheumatic joint pain (Kirtikar and Basu, 2002). The bark is also used to treat bronchitis and snakebite (Rani et al., 2012; Aggarwal et al., 2011; Chatterjee et al., 2007). The plant seed powder is used for scalp scurvy, alopecia and as an anthelmintic agent (Chatterjee et al., 2007; Nair et al., 2005). The seed powder

A phytochemical investigation was conducted on the flowers of *Nyctanthesarbor-tristis* Linn. For isolation of compounds, the dried flower's powder was successively extracted with n-hexane, dichloromethane, ethyl acetate and methanol. The extracts were fractionated using different chromatographic techniques and four compounds were isolated. Stigmasterol (1) from n-hexane, rengyolone (2) from dichloromethane and two other compounds namely, 2-phenylethyl  $\beta$ -D-glucopyranoside (3) and n-tetradecyl- $\beta$ -D-glucopyranoside (4) from ethyl acetate extract, were isolated. These compounds (1-4) were characterized on the basis of IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, DEPT-135 NMR. Compounds 1 and 3 were isolated for the first time from this plant while compound 4 has been isolated and completely characterized from this plant as well as from the natural sources.

**Keywords**: Phytochemical investigation; *Nyctanthes arbor-tristis* Linn; Stigmasterol; Rengyo- lone; 2-Phenylethyl β-D-glucopyranoside; n-Tetradecyl-β-D-glucopyranoside

is also used in piles and skin diseases (Kirtikar and Basu, 2002). Among other medicinal uses, the root of the plant is used for the treatment of fever, sciatica and anorexia (Rani *et al.*, 2012). The flowers are used as stomachic, carminative, astringent to bowels, antibilious expectorant, and in the treatment of piles and various skin diseases (Rani *et al.*, 2012). The flower juice is used as a tonic for preventing graying of hairs and hair fall (Girach *et al.*, 1994). The previous phytochemical investigations of this plant resulted in the isolation of nyctantic acid, friedelin,  $\beta$ -sitosterol, oleanolic acid (Rout *et al.*, 2007), arbortristoside-A,  $\beta$ -amyrin, nyctoside-A, and 6- $\beta$ -hydroxyloganin (Rathore *et al.*, 2007).

In this study, n-hexane, dichloromethane and ethyl acetate extracts of *Nyctanthes arbor-tristis* Linn flowers were subjected to extensive chromatographic separation to afford a steroid stigmasterol (1), benzofuranonerengyolone (2), and two  $\beta$ - D- glucopyranosides 2- phenylethyl  $\beta$ -D-glucopyranoside (3) and n-tetradecyl- $\beta$ -D- glucopyranoside (4) (Fig. 1). These compounds (1-4) were characterized on



Fig. 1. Structures of compounds 1-4, isolated from the flowers of Nyctanthes arbor-tristis Linn

the basis of spectroscopic data (IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR and DEPT-135 NMR) and elemental analysis.

### Materials and methods

### General experimental procedures

Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were performed using pre-coated aluminum sheets and glass plates with silica gel  $60F_{254}$ (Merck, Darmstadt, Germany), whereas the columns were wet packed with silica gel (0.063-0.20 mm, silica gel 60, AppliChem, Darmstadt, Germany) and vacuum liquid chromatography (VLC) column was packed with TLC grade silica gel (60H, 0.045mm, Merck, Darmstadt, Germany). NMR spectra were recorded on a Bruker ASCEND<sup>TM</sup> 400 MHz (1H) and 100 MHz (13C and DEPT-135) spectrometer. IR spectra were recorded on an IR Affinity-1S SHIMADZU FT-IR spectrophotometer. Melting points were determined on an electro-thermal melting point apparatus (Stuart Scientific SMP3, UK) and are uncorrected. All of the solvents and chemicals used for this research were analytical reagent grade, procured from E. Merck (Germany), BDH (England), AppliChem (Germany) and Sigma Aldrich (Germany).

### Plant materials

The flowers of *Nyctanthes arbor-tristis* Linn were collected from BCSIR campus, Dhaka, Bangladesh, in October 2013. The plant (specimen # DACB 38734) was identified from Bangladesh National Herbarium (BNH), Dhaka by their Taxonomist. The collected flowers were air dried at 25-30°C in the absence of sunlight. The dried flowers were powdered by a grinder. Then they were weighed and stored in an air tight container in dark until use.

### Extraction and isolation

The dried powdered flower (265.12 g) was extracted successively with n-hexane, dichloromethane, ethyl acetate and methanol at room temperature. Extractions were carried out three times with each solvent and the solvent was evaporated using a vacuum rotary evaporator (BUCHI, Switzerland) at low temperature (below 40 °C) under reduced pressure.

# Chromatographic separation of n-hexane extract: isolation of compound 1

4.89 g of the crude n-hexane extract was purified by repeated column chromatography, eluting first with 100% n-hexane and then with n-hexane and dichloromethane mixtures of different ratios and finally with 100% methanol to give 27

fractions. The fractions were combined by their behavior on TLC. Fractions 11-14, MMH-1 (yield 1.575 g) was further chromatographed over a AppliChem silica gel column (0.063-0.20 mm). The column was first eluted with  $CH_2Cl_2$ : n-hexane, 7:3, v/v and then with increasing polarity of solvent, and then with 100% dichloromethane and finally with 50% methanol in  $CH_2Cl_2$ . Eluates were collected in a series of 33 fractions and were combined based on TLC results. Fractions14-18 were identified as MMH-2 (0.2371 g) which was further separated by column chromatography eluting first with 100% n-hexane and then solvent mixtures with increasing polarity and finally with 5% ethyl acetate in  $CH_2Cl_2$ . Eluents were collected in a series of 17 fractions. Fractions 5-11 yielded white needle shaped crystals, identified as MMH-3 (compound 1, 0.048 g).

Analytical and spectral data for 1: White needle shaped crystals; Mp. 167-169 °C (Lit. 169-171 °C) (Koay *et al.*, 2013). IR (cm<sup>-1</sup>): 3238, 2934, 2864, 1651, 1454, 1445, 1051, 970. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  0.68 (s, 3H), 0.80 (d, 3H), 0.81 (t, 3H), 0.83 (d, 3H), 0.99 (s, 3H), 1.02 (d, 3H), 3.51 (m, 1H), 5.01 (dd, *J* = 15.2, 8.8 Hz, 1H), 5.14 (dd, *J* = 15.2, 8.4 Hz, 1H), 5.34 (m, 1H).<sup>13</sup> C NMR (CDCl<sub>3</sub>):  $\delta$  37.3, 31.6, 71.8, 42.2, 140.8, 121.7, 31.9, 51.2, 36.5, 21.1, 39.7, 42.2, 56.9, 24.4, 28.9, 56.0, 12.0, 19.4, 40.5, 21.2, 138.3, 129.3, 51.2, 31.6, 21.2, 19.0, 25.4, 12.3.

Chromatographic separation of dichloromethane extract: isolation of compound 2

7.74 g of the dichloromethane extract was purified by repeated column chromatography, eluting first with 100% n-hexane and then with increasing polarity with dichloromethane of different ratios and finally with 100% methanol. Fractions 18 and 19 were combined (MMH-4, 4.97 g). This fraction was further subjected to column chromatography. The column was first eluted with 50% CH<sub>2</sub>Cl<sub>2</sub> in n-hexane and then with increasing polarity with CH<sub>2</sub>Cl<sub>2</sub>, finally with 100% CH<sub>2</sub>Cl<sub>2</sub> and then with 20% methanol (MeOH) in CH<sub>2</sub>Cl<sub>2</sub>. Eluates were collected in 27 fractions. Fractions 22-24 were combined and identified as fraction MMH-5 (1.6g). This fraction was further subjected to column chromatography. The column was first eluted with 100% CH<sub>2</sub>Cl<sub>2</sub> and finally with 20% methanol (MeOH) in dichloromethane. Eluents were collected in a series of 42 fractions. The fractions 26-38 were combined and identified as fraction MMH-6 (0.4648 g). This fraction was subjected to PTL on pre-coated glass plate sheets with appropriate ratio of selected solvent system (CH<sub>2</sub>Cl<sub>2</sub>: MeOH 95:5 v/v). The individual bands on the plates were scraped with the help of a spatula and were extracted several times with 20% MeOH in CH<sub>2</sub>Cl<sub>2</sub>. Excess solvent was evaporated by using a rotary

evaporator. Compound 2 (0.0969 g, soluble in  $CH_2Cl_2$ ) was finally obtained as colorless oil.

Analytical and spectral data for 2: Colorless oil; boiling point 330-333°C (Lit. 332.9 °C) (Siddiqui *et al.*, 2007; Endo and Hikino, 1984). IR (cm<sup>-1</sup>): 3391, 1667, 1447, 1128, 1061 and 1015. <sup>1</sup>H NMR (CDCl<sub>3</sub>):  $\delta$  6.71 (dd, J = 10.4, 1.5 Hz, 1H), 5.92 (d, J = 10.4 Hz, 1H), 4.15 (ddd, J = 4.4 Hz, 1H), 4.0 (ddd, J = 7.2, 7.6, 6.0Hz, 1H), 3.87 (ddd, J = 7.6, 7.6, 8.0 Hz, 1H), 2.73(dd, J = 4.0, 16.8 Hz, 1H), 2.54 (dd, J = 5.2, 17.2 Hz, 1H), 2.28 (ddd, J = 6.4, 7.2, 13.6 Hz, 1H), 2.16 (ddd, J = 6.4, 7.2, 13.6 Hz, 1H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>):  $\delta$  75.1 (C-1), 81.2 (C-2), 39.9 (C-3), 197.5 (C-4), 128.2 (C-5), 148.7 (C-6), 39.3 (C-7), 66.1 (C-8).

# Chromatographic separation of ethyl acetate extract: isolation of compound 3

7.95 g of the ethyl acetate extract was subjected to vacuum liquid chromatography (VLC). The VLC column was first eluted with 100% n-hexane and then with increasing polarity with ethyl acetate-n-hexane and ethyl acetate-methanol of different ratios and finally with 100% MeOH. Eluates were collected in a total of 15 fractions. The fractions of similar R<sub>c</sub> were combined as MMH-7 (4.3173 g) which was further subjected to column chromatography. The column was first eluted with 100% CH<sub>2</sub>Cl<sub>2</sub> and then with CH<sub>2</sub>Cl<sub>2</sub> and methanol mixtures of different ratios and finally with 40% methanol in CH<sub>2</sub>Cl<sub>2</sub>. Eluates were collected in 11 fractions. The fraction MMH-8 (1.6450 g) was further subjected to column chromatography. The column was first eluted with 5% methanol in CH<sub>2</sub>Cl<sub>2</sub> and then with increasing the polarity of CH<sub>2</sub>Cl<sub>2</sub> and methanol mixtures of different ratios and finally with 30% methanol in CH<sub>2</sub>Cl<sub>2</sub>. Eluents were collected in a series of 98 fractions. The fractions 32-41 combined as MMH-9 and fractions 53-66 were combined as fraction MMH-10 (0.6513 g). The fraction MMH-9 was further subjected to column chromatography. The column was eluted with 2% methanol in ethyl acetate while a total of 33 fractions were collected. The fractions of similar spots in TLC were combined together and designated MMH-11 which was further identified as compound 3 (0.0868 g).

Analytical and spectral data for 3: Colorless crystalline solid; Mp. 37-39 °C (Lit. 38.5 °C) (Joshi and Sawant, 2006). IR (cm<sup>-1</sup>): 3354, 2880, 1454,1072, 1016. <sup>1</sup>H NMR (CD<sub>3</sub>OD): δ 7.21 (m, 5H), 4.28 (d, J = 7.6 Hz, 1H), 4.07 (m, 2H), 3.82 (td, J = 12.0, 2.4 Hz, 1H), 3.73 (dd, J = 5.6, 11.6 Hz, 1H), 3.69 (dd, J = 4.8, 12.0 Hz, 1H), 3.38 (m, 1H), 3.29 (m, 1H), 3.21 (ddd, J = 2.0, 7.2, 9.2 Hz, 1H), 2.91 (t, J = 6.8 Hz, 2H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ 138.3 (C-1), 128.3 (C-2, C-6), 128.8 (C-3, C-5), 126.2 (C-4), 102.9 (C-1), 73.5 (C-2), 76.4 (C-3), 70.0 (C-4), 76.0 (C-5), 61.5 (C-6), 36.0 (C-α), 70.6 (C-β). Chromatographic separation of ethyl acetate extract: isolation of compound 4

The fraction MMH-10 (0.6513 g) was separated by carefully decanting of the mother liquor from the test tube. The fraction was washed with n-hexane and then n-hexane-CH<sub>2</sub>Cl<sub>2</sub> mixture, finally using one or two drops of methanol. The crystalline fraction was further subjected to column chromatography. The column was first eluted with 100% ethyl acetate and then with increasing the polarity of solvents of ethyl acetate and methanol mixtures of different ratios and finally 30% methanol in ethyl acetate. Eluates were collected in a series of 9 fractions. The fractions 5-6 was obtained colorless powder and identified as fraction MMH-12 which shows a single spot on TLC. This fraction was identified as compound 4 (0.0012 g, soluble in methanol).

Analytical and spectral data for 4: Colorless powder; Mp. 120–122 °C (Lit. 119–121 °C) (Tickle *et al.*, 1998). IR (KBr, cm<sup>-1</sup>): 2918, 1081. <sup>1</sup>H NMR (CD<sub>3</sub>OD):  $\delta$  4.26 (d, *J* = 6.8 Hz, 1H), 4.07 (m, 2H), 3.87 (dd, *J* = 5.9, 11.6 Hz, 1H), 3.67 (dd, *J* = 4.8, 12.0 Hz, 1H), 3.60 (dd, *J* = 6.4, 12.4 Hz, 1H), 3.52 (m, 1H), 3.27 (d, *J* = 6.4 Hz, 1H), 3.17 (t, *J* = 8.0 Hz, 1H), 1.61 (m, 2H), 1.29 (m, 22H), 0.90 (t, *J* = 6.4, 13.6 Hz, 3H). <sup>13</sup>C-NMR (CDCl<sub>3</sub>):  $\delta$  69.9 (C-1), 35.7 (C-2), 33.7 (C-3), 33.1 (C-4), 30.8 (C-5), 30.8 (C-6), 30.7 (C-7), 30.7 (C-8), 30.5 (C-9), 30.5 (C-10), 28.3 (C-11), 26.1 (C-12), 23.7 (C-13), 14.4 (C-14), 104.7 (C-1), 75.0 (C-2), 78.0 (C-3), 71.5 (C-4), 75.5 (C-5), 62.6 (C-6).

#### **Results and discussion**

After fractionation and purification, the structure elucidation of the four compounds isolated from n-hexane (compound 1), dichloromethane (compound 2) and ethyl acetate extracts (compounds 3 and 4) of *Nyctanthes arbor-tristis* Linn flowers (Fig. 1) was carried out by spectral analysis.

### Structural identification for compound 1

The compound 1 showed positive tests for steroids and alcohols. The IR spectrum exhibits the band of a hydroxyl group at 3238 cm<sup>-1</sup> and two sharp absorptions at 2934 and 2864 cm<sup>-1</sup> for C-H stretching for sp<sup>3</sup> hybridized aliphatic carbon. The spectrum also shows absorptions at 1651 (C=C stretching, sp<sup>2</sup>), 1454 (C-H bending CH<sub>2</sub> group), and 1445 cm<sup>-1</sup> (C-H bending CH<sub>3</sub> group). The absorption at 1051 cm<sup>-1</sup> is due to C-O stretching (primary alcohol) while the absorption at 970 cm<sup>-1</sup> is due to C-H bending (trans alkene). The <sup>1</sup>H-NMR spectrum exhibits a multiplet at  $\delta$  3.51

corresponding to one proton, whose position and multiplicity corresponds to the H-3 of the steroid nucleus. A multiplet at  $\delta$  5.34, integrating for 1H, is indicative of the typical signal for the olefinic H-6 of the steroid nucleus (steroid skeleton). The down-field resonances at  $\delta$  5.14 (dd, J = 15.2, 8.4 Hz, 1H) and 5.01 (dd, J = 15.2, 8.8 Hz, 1H) are assigned to olefinic protons (H-22 and H-23), respectively. The spectrum also contains two singles at  $\delta$  0.68 and 0.99 (integrating to 3H each), assigned to two tertiary methyl groups at C-18 and C-19, respectively. The doublets at  $\delta$  0.80 and 0.83 (integrating to 3H each) are due to two methyl groups at C-26 and C-27 and the doublet at  $\delta$  1.02 (d, 3H) is assigned to the methyl group at C-21. On the other hand, a triplet at  $\delta$  0.81 (integrating to 3H) is due to the primary methyl group at C-29. The <sup>13</sup>C NMR spectrum shows the presence of 29 carbon atoms. The signal at  $\delta$  71.8 at C-3 carbon atom is characteristics of  $\beta$  - hydroxyl group. The resonances at  $\delta$ 138.3 and 129.3 correspond to a double bond at C-22 and C-23, respectively. The signals at  $\delta$  140.8 and 121.7 for C-5 and C-6 are indicative of another double bond. However, resonances at  $\delta$  12.0 and 19.4 represent angular methyl carbon atoms for C-18 and C-19, respectively.

### Structural identification for Compound 2

Functional group test showed positive tests for hydroxyl group. The IR spectrum of this compound exhibits a broad absorption band at 3391 cm<sup>-1</sup> (characteristic of O-H stretching) and an absorption at 1667 cm<sup>-1</sup> (characteristic of C = Ostretching). The absorptions at 1061 and 1015 cm<sup>-1</sup> are due to C-O stretching of primary alcohol. The absorption at 1447  $cm^{-1}$  is assigned to aromatic C = C stretching, while that at 1128 cm<sup>-1</sup> is due to C-O stretching of C-O-C group. The <sup>1</sup>H-NMR spectrum reveals the presence of an oxymethylene group ( $\delta$  4.01, ddd, J = 7.2, 7.6 Hz and  $\delta$  3.87, ddd, J = 7.6, 8.0, 7.6 Hz, 1H,) adjacent to a methylene group ( $\delta$  2.28, ddd, J = 6.4, 7.2, 13.6 Hz, 1H and  $\delta$  2.16, ddd, J = 6.4, 7.2, 13.6 Hz, 1H), an oxymethine group ( $\delta$  4.15, ddd, J = 4.4 Hz, 1H) next to another methylene group ( $\delta$  2.54, dd, J = 5.2, 17.2 Hz;  $\delta$  2.73, 1H, dd, J = 4, 16.8 Hz, 1H), and an  $\alpha$ ,  $\beta$ -unsaturated carbonyl group ( $\delta$  5.92, 1H, d, J = 10.4 Hz; 6.71, 1H, dd, J = 10.4 Hz). The <sup>13</sup>C-NMR spectrum showed the presence of eight carbon atoms (δ 197.5, 148.7, 128.2, 81.2, 75.1, 66.1, 39.9 and 39.3). In DEPT-135 spectrum, three signals ( $\delta$  148.7, 128.2 and 81.2) are as positive only for methine (CH) carbon atoms and three signals ( $\delta$  66.1, 39.9 and 39.3 ppm) are negative signals for methylene (CH<sub>2</sub>) carbon atoms. Two quaternary signals



Fig. 2. <sup>13</sup>C{<sup>1</sup>H} and DEPT-135 NMR spectra of compound 2

appeared at  $\delta$  197.5 and 75.1 of which the former corresponds to a ketonic carbon (C-4). <sup>13</sup>C{<sup>1</sup>H} and DEPT-135 NMR spectra of compound **2** are provided in Fig. 2.

### Structural identification for Compound 3

The IR spectrum of this compound showed a broad absorption band at 3354 cm<sup>-1</sup>, characteristic of O-H stretching and absorption at 2880 cm<sup>-1</sup>, characteristic of C-H stretching (aliphatic, sp<sup>3</sup> carbon). The absorption band at 1016 cm<sup>-1</sup> was due to C-O stretching (primary alcohol). Other absorptions were at 1454 cm<sup>-1</sup> (C = C stretching, aromatic) and 1072 cm<sup>-1</sup> (C-O stretching, C-O-C). The <sup>1</sup>H-NMR spectrum of compound **3** showed a multiplet at  $\delta$  7.21 (m, 5H) due to aromatic protons of the benzene ring at C-2, C-3, C-4, C-5 and C-6, and a triplet at  $\delta$  2.91 (t, J = 6.8 Hz, 2H) due to the benzylic methylene protons at C- $\alpha$ . A doublet at  $\delta$  4.28 (1H, J = 7.6 Hz) indicated a  $\beta$ -anomeric proton of glycoside moiety. The <sup>13</sup>C-NMR spectrum revealed the presence of 14 carbon atoms. Signals of these carbon atoms

and 36.0 ppm) are negative signals for methylene (CH<sub>2</sub>) carbon atoms. There is one quaternary signal at  $\delta$  138.3 ppm (C-1). The <sup>13</sup>C-NMR and DEPT-135 NMR spectra of compound **3** indicated the presence of four oxymethine carbons with chemical shift between  $\delta$  70.0 and 76.4 ppm, one methylene carbon signal at  $\delta$  61.5 and one methine carbon signal at  $\delta$  102.9 (C-1) due to an anomeric carbon of a glycoside. This evidence clearly indicates the presence of a glucopyranosyl group. The <sup>13</sup>C-NMR and DEPT-135 NMR spectra of this compound also indicated the presence of another two methylene carbon signals at  $\delta$  70.6 (C- $\beta$ ) and 36.0 ppm (C- $\alpha$ ). <sup>13</sup>C{<sup>1</sup>H} and DEPT-135 NMR spectra of compound 3 are provided in Fig. 3.

### Structural identification for Compound 4

The IR spectrum of this compound exhibits absorption bands at 2918 cm-1 (C-H stretching, aliphatic, sp3) and 1081 cm<sup>-1</sup> (C-O stretching, C-O-C). The <sup>1</sup>H-NMR spectrum of compound 4 exhibits a triplet at  $\delta$  0.90 (J = 13.6 Hz) which



Fig. 3. <sup>13</sup>C{<sup>1</sup>H} and DEPT-135 NMR spectra of compound 3

were at  $\delta$  138.3, 128.8, 128.8, 128.3, 128.3, 126.2, 102.9, 76.4, 76.0, 73.5, 70.6, 70.0, 61.5, and 36.0 ppm. In DEPT-135 spectrum, ten signals ( $\delta$  128.8, 128.8, 128.3, 128.3, 126.2, 102.9, 76.4, 76.0, 73.5 and 70.0 ppm) are as positive only for methine (CH) carbon atoms and three signals ( $\delta$  70.6, 61.5, was due to the primary methyl group at C-14, a methylene proton signal at  $\delta$  1.29 (m, C3 - C13, 22H) was due to a long chain, suggesting that this compound had an n-alkyl group. A doublet at  $\delta$  4.26 (d, J = 6.8 Hz, 1H) indicated a  $\beta$ -anomeric proton of glycoside moiety. The 13C-NMR spectrum showed

the presence of 20 carbon atoms in total. The resonance of these carbon atoms are at  $\delta$  104.7, 78.0, 75.5, 75.0, 71.5, 69.9, 62.6, 35.7, 33.7, 33.1, 30.8, 30.8, 30.7, 30.7, 30.5, 30.5, 28.3, 26.1, 23.7 and 14.4 ppm. In DEPT-135 spectrum the positive signal at  $\delta$  14.4 ppm is assigned to methyl (CH3) carbon atoms, and the five positive signals at  $\delta$  104.7, 78.0, 75.5, 75.0 and 71.5 ppm are assigned to methine (CH) carbon atoms and fourteen signals at  $\delta$  69.9, 62.6, 35.7, 33.7, 33.1, 30.8, 30.8, 30.7, 30.7, 30.5, 30.5, 28.3, 26.1 and 23.7 ppm were negative signals for methylene (CH2) carbon atoms. There are no quaternary signals for this compound. The <sup>13</sup>C-NMR and DEPT-135 NMR spectra of compound 4 indicated the presence of four oxymethine carbons with chemical shift between  $\delta$  71.5 and 78.0 ppm, one methylene carbon signal at  $\delta$  62.6 and one methine carbon signal at  $\delta$ 104.7 (C-1) due to an anomeric carbon of a glycoside. Evidences clearly indicate that it is a substituted glucopyranosyl molecule.

### Conclusions

In summary, the spectroscopic data of compound 1 is in complete agreement to those reported for stigmasterol (Nayak et al., 2015; Koay et al., 2013; Jamal et al., 2008; Jain and Bari, 2010). The spectroscopic data of compound 2 is in complete agreement to the data reported for rengyolone. (Siddiqui et al., 2007; Endo and Hikino, 1984). For the compound 3 on the other hand, the physical and spectral data was in complete agreement to the reported data in literature (Joshi and Sawant, 2006; Kim et al., 2008; Schwab and Schreier, 1988; Umehara et al., 1988). Thus compound 3 has been characterized as 2-phenylethyl β-D-glucopyranoside. It is also concluded that two compounds (1 and 3) were isolated for the first time from this plant. The compound 4 has been characterized as n-tetradecyl-\beta-D-glucopyranoside (Figure 1) and the physical and spectral data is in complete agreement with the data reported in the literature (Tickle et al., 1998; Joshi and Sawant, 2006). Compound 4 has been isolated and completely characterized from this plant as well as from other natural sources.

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