New lipoxygenase and cholinesterase inhibitory sphingolipids from *Carthamus oxyacantha*

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**1. Introduction**

The genus *Carthamus* of the plant family ‘Asteraceae’ comprises 25 species which are very important as pharmaceutical point of view and have commercial values as well (Hacioglu et al. 2012; Talebi et al. 2012). *Carthamus tinctorius* is used for the treatment of cough, typhoid

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**ABSTRACT**

Two new sphingolipids: oxyacanthin A [(25,35,4R)-2-[[2R,5E]-2-hydroxyoctadec-5-enoyl]amino]hexaeicosane-1,3,4-triol; 1] and B [(25,35,4R)-2-[[2R,5E]-2-hydroxyoctadec-5-enoyl]amino]hexaeicosane 1,3,4-triol-1-O-β-D-glucopyranoside; 2], together with 1-octacosanol, β-sitosterol, β-sitosterol 3-O-β-D-glucopyranoside and luteolin 7-O-β-glucopyranoside were isolated from the methanolic extract of the whole plant of *Carthamus oxyacantha*. Their structures were elucidated using 1H and 13C NMR spectra and 2D NMR analyses (HMQC, HMBC and COSY) in combination with mass spectrometry (EI-MS, HR-EI-MS, FAB-MS and HR-FAB-MS) experiments and in comparison with the literature data of the related compounds. Both the compounds 1 and 2 showed inhibitory potential against lipoxygenase (LOX) in a concentration-dependent manner with IC50 values 83.3 ± 1.3 and 245.7 ± 1.1 µM, whereas compound 2 showed inhibition against enzymes acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) with IC50 values 65.3 ± 0.1 and 93.6 ± 0.1 µM, respectively.

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**KEYWORDS**

*Carthamus oxyacantha*; sphingolipids; secondary metabolites; lipoxygenase inhibition; cholinesterase inhibition

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fever, throat disorders and as herbal tea to reduce blood cholesterol levels (Khan & Khatoon 2008). It is also used to get relief from cardiovascular problems, swelling, menstrual disorders and pain (Arpornsuwan et al. 2010). It shows anti-apoptotic, anti-inflammatory, antioxidant and anti-thrombotic activities (Jun et al. 2011). In China, Japan, India and Korea, it is used as herbal medicine for the treatment of inflammation, gynecological disorders and heart problems (Arpornsuwan et al. 2012). It also acts as neuroprotective, calcium antagonist and anti-coagulant (Han et al. 2009). The decoction of C. tinctorius is used to reduce blood viscosity (Li et al. 2009). The oil of its seeds contains linoleic acid and various amount $\alpha$-tocopherol, $\beta$-tocopherol and $\gamma$-tocopherol which are helpful in reducing blood cholesterol levels (Matthaus et al. 2015). The extract of C. lanatus possesses anti-inflammatory, anti-microbial and anti-analgesic activities (Topashka-Ancheva et al. 2006) and also used traditionally as sedative and anti-tumour agent (Taskova et al. 2002; Bocheva et al. 2003). Carthamus oxyacantha is found in dry places and plains of Pakistan, Afghanistan, Turkmenistan, India, Azerbaijan, Iraq, Tajikistan, Kyrgyzstan and Iran. The flowers of C. oxyacantha are used as herbal remedy in rheumatism, cerebral thrombosis, bronchitis, male infertility and jaundice, while its seeds have laxative property and oil is used in scabies therapy. It is used to reduce pain and swelling in injuries, exhibits labour induction property, increases blood circulation (Ahmad et al. 2007–2010) and has anti-hyperlipidemic character (Ahmad et al. 2009). The diverse biological activities of C. oxyacantha motivated us to explore this plant for its bioactive constituents. Herein, we report the isolation and characterisation of two new sphingolipids (1, 2) (Figure 1) together with 1-octacosanol (Safder et al. 2009), $\beta$-sitosterol (Rubinstein et al. 1976; Bernard & Eokes 1977; Holland et al. 1978), $\beta$-sitosterol 3-$\beta$-D-glucopyranoside (Rubinstein et al. 1976; Bernard & Eokes 1977; Holland et al. 1978) and luteolin 7-$\beta$-D-glucopyranoside (Wang et al. 1998) from the methanolic extract of the whole plant of C. oxyacantha.

Sphingolipids are the waxy lipids composed of sphingosine and a fatty acid generally found in high concentrations within the cell membrane of cells. They are one of the component lipids that make up sphingomyelin, one of the major lipids in the lipid bilayer. Sphingolipids can participate in a variety of cellular signalling including regulating differentiation, proliferation and programmed cell death of cells. They are implicated in a variety of physiological functions including apoptosis, cell growth arrest, differentiation, cell senescence, cell migration and adhesion. Roles for these chemicals and their downstream metabolites have also been suggested in a number of pathological states including cancer, neurodegeneration, diabetes, microbial pathogenesis, obesity and inflammation (Hannun & Obeid 2008).

![Figure 1](image.png)

**Figure 1.** Structures of compounds 1 and 2 isolated from C. oxyacantha.
Lipoxygenases (LOX, EC 1.13.11.12) are potential targets for the rational drug design and discovery of mechanism-based inhibitors for the treatment of a variety of disorders such as bronchial asthma, inflammation, cancer and autoimmune diseases. Cholinesterase enzymes (AChE, BChE) are implicated as key biological players in Alzheimer’s disease, which makes them logical targets for inhibitory therapeutics. Compounds 1 and 2 were evaluated for LOX, AChE and BChE inhibitory potential using baicalein and eserine (Aldrich Chem. Co.; Seelze, Germany) as positive controls in the assays. The results (Table 1) showed that compounds 1 and 2 were moderate inhibitors of LOX, whereas compound 2 showed moderate to weak inhibitory potential against AChE and BChE.

2. Results and discussion

Compound 1 was isolated as colourless amorphous powder. Its IR spectrum showed the absorption bands at 3500 (O–H), 3450 (N–H), 1670 (C=O) and 1650 (C=C) cm⁻¹. The HR-EI-MS showed the molecular ion peak [M]+ at m/z 709.6567 corresponding to the molecular formula C₄₄H₈₇NO₅. The ¹H NMR spectrum of 1 afforded a signal at δ_H 7.80 (d, J = 8.8 Hz, 1H) was attributed to an amide function. Two olefinic protons were observed as a broad multiplet at δ_H 5.32 (br m, 2H). The multiplicity of this signal at half height was calculated to be 22.5 Hz. The W₁/₂ (22.5 Hz) and the chemical shifts of C-4′ and C-7′ (δ 35.5, 33.8) adjacent to the double bond indicated its trans geometry of the double bond (Shibuya et al. 1990). A methine resonating at δ_H 3.91 (m, 1H) was correlated in HSQC spectrum with a carbon at δ_C 51.5 revealed its attachment with the amide function. Furthermore, the same spectrum showed three oxymethines at δ_H 3.82 (dd, J = 6.6, 3.4 Hz, 1H), 3.46 (dd, J = 4.1, 3.7 Hz, 1H), 3.38 (m, 1H) and an oxymethylene at δ_H 3.63 (dd, J = 11.5, 3.8 Hz, 1H) and 3.57 (dd, J = 11.5, 5.0 Hz, 1H). The ¹³C NMR spectrum of 1 (Table 1) displayed signals at δ_C 173.2 and 131.4 for carbonyl and two olefinic carbons, three oxymethines at δ_C 75.1 (C-3), 72.9 (C-2′) and 72.7 (C-4) and an oxymethylene at δ_C 60.6 (C-1), respectively. Another methine carbon resonating at δ_C 51.5 (C-2) was attested for an azomethine function. These values were similar to the data reported for ceramides (Kwon et al. 2003; Gao et al. 2004; Riaz et al. 2012) concluding 1 to be a ceramide. The lengths of fatty acid and amine chains could be determined due to mass spectrometry and/or chemical degradation. The length of the fatty acid chain was found to be composed of eighteen carbons due to a fragment ion at m/z 281 in EI-MS spectrum containing double bond and a hydroxyl group. The position of the double bond could be determined due to COSY spectrum, fragment ions at m/z 195 and 169 and HMBC correlations. On the other hand, the length of the amine chain was also determined due to fragment ion in EI-MS spectrum at m/z 428. Two hydroxyl groups were confirmed at C-3 and C-4 due to COSY correlations of azomethine (δ_H 3.91, H-2) with oxymethylene (δ_H 3.63, 3.57, H-1) and oxymethylene (δ_H 3.46, H-3) which further correlated with H-4 (δ_H 3.38). Methanolation of 1 followed by acetylation and GC–MS analysis indentified

Table 1. AChE, BChE and LOX inhibition studies of compounds 1 and 2.

| Sr. no. | Conc. (mM) | % inhibition | IC₅₀ (µM) | % inhibition | IC₅₀ (µM) | % inhibition | IC₅₀ (µM) |
|---------|------------|-------------|----------|-------------|----------|-------------|----------|
| 1       | 0.5        | 7.6 ± 0.1   | 16.7 ± 0.1 | 84.2 ± 1.6 | 83.3 ± 1.3 |
| 2       | 0.5        | 84.1 ± 0.3  | 58.1 ± 0.2 | 93.6 ± 0.1 | 57.8 ± 1.2 | 245.7 ± 1.1 |
| Eserine | 0.5        | 91.3 ± 1.1  | 82.8 ± 1.1 | 93.8 ± 1.3 | 22.4 ± 1.3 |

Notes: Compounds were dissolved in methanol and assayed at concentration of 0.5 mM. Results are presented as mean ± sem, n = 3.
the fatty acid and amine chains as methyl 2-acetoxyoctadec-5-enoate (m/z 354) and 2-acetamino-1,3,4-triacetoxyhexaeicosane (m/z 597), respectively. Position of the double bond was further confirmed through its cleavage by periodate (Ahmed et al. 2007). It is reported in the literature that both the optical rotation and the chemical shifts of both the 1H and 13C atoms at the stereogenic centres provide a strong evidence for the absolute configuration of sphingolipids. Since most of the spectra of sphingolipids have so far been reported in pyridine, therefore, the 1H NMR spectrum of 1 was repeated in pyridine, and the chemical shifts of the H-atoms attached to the stereogenic were found to be \( \delta_H \) 4.57 (dd, \( J = 10.4, 5.3 \) Hz, H-1a, 1H), 4.40 (dd, \( J = 10.4, 3.1 \) Hz, H-1b, 1H), 5.08 (m, H-2, 1H), 4.32 (dd, \( J = 4.7, 3.2 \) Hz, H-3, 1H), 4.25 (td, \( J = 5.5, 4.7 \) Hz, H-4, 1H) and 4.56 (t, \( J = 6.5 \) Hz, H-2′, 1H), whereas the carbons shifts of the respective centres were appeared at \( \delta_C \) 61.4, 52.0, 75.5, 72.4 and 72.7, respectively, which were in complete agreement with those of sphingolipids with a 2S,3S,4R,2′R configuration (Muralidhar et al. 2005; Kang et al. 2007; Riaz et al. 2007; Dongfack et al. 2012). The optical rotations of 1 ([\( \alpha \]D] = +41.0) and its methanolysis products ([\( \alpha \]D] = 8.1 and +18.9) which were comparable with those of sphingolipids with a 2S,3S,4R,2′R configurations (Muralidhar et al. 2005; Kang et al. 2007; Dongfack et al. 2012). Based on the above data and discussion, compound 1 was finally characterised as (2S,3S,4R)-2-[(2R,5E)-2-hydroxyoctadec-5-enoyl]amino]hexaeicosane-1,3,4-triol.

Compound 2 was isolated as colourless gummy solid. Its IR spectrum displayed absorption band at 3505 (O–H), 3440, 1670 (amide) and 1655 cm \(^{-1} \) (C=C) similar to that of 1. The HR-FAB-MS showed molecular ion peak [M–H] + at m/z 870.7021 gave molecular formula C\(_{50}\)H\(_{96}\)NO\(_{10}\). The 1H and 13C NMR spectra of 2 were very similar to that of 1 containing the usual signals for ceramide moiety, with the addition of an anomeric methine at \( \delta_H \) 4.28 (d, \( J = 7.5 \) Hz, H-1″, 1H) indicating the presence of a sugar moiety in 2. The remaining signals for sugar unit were observed at \( \delta_H \) 3.85 (dd, \( J = 10.4, 4.5 \) Hz, H-6″a, 1H), 3.71 (dd, \( J = 10.4, 2.9 \) Hz, H-6″b, 1H), 3.79 (m, H-5″, 1H), 3.65 (m, H-4″, 1H), 3.39 (m, H-2″, 1H) and 3.36 (m, H-3″, 1H). The 13C NMR spectrum of 2 also provided evidence for ceramide glycoside as it displayed anomeric carbon at \( \delta_C \) 104.7 (C-1″) with other sugar signals at \( \delta_C \) 78.0 (C-5″), 77.9 (C-2″), 72.9 (C-3″), 71.5 (C-4″) and a methylene carbon at \( \delta_C \) 62.6 (C-6″) (Riaz et al. 2007, 2012). The attachment of sugar could be confirmed at C-1 due to its downfield shift at \( \delta_C \) 69.9 and HMBC correlation of H-1″ (\( \delta_H \) 4.28) with C-1 (\( \delta_H \) 69.9). The length of fatty acid and amine chains, position of the double bond and hydroxyl groups were found similar as in 1 by comparing the mass fragmentation patterns, COSY spectrum and HMBC correlation and by chemical methods (methanolysis and periodate cleavage) of 2 and 1, respectively. The stereochemistry at various stereogenic centres was found similar to those for 1. Based on the above data and discussion, compound 2 has been found to be glucoside of 1 and characterised as [(2S,3S,4R)-2-([(2R,5E)-2-hydroxyoctadec-5-enoyl]amino]hexaeicosane-1,3,4-triol-1-O-β-D-glucopyranoside.

3. Experimental

3.1. General experimental procedures

Infrared (IR) spectra were recorded on Shimadzu 460 spectrometer (Duisburg, Germany). 1H NMR, HMQC, HMBC and COSY spectra were acquired on either a 400 or 500 MHz, whereas the 13C NMR spectra were recorded on a 100 or 125 MHz on Bruker spectrometer (Zurich, Switzerland). The chemical shift values (\( \delta \)) are reported in ppm, and the coupling constant (\( J \)) is in Hz. EI-MS, HR-EI-MS, FAB-MS and HR-FAB-MS were recorded on Finnigan (Varian MAT) JMS H × 110 with a data system and JMSA 500 mass spectrometers (Varian MAT, Waldbronn,
Germany), respectively. The gas chromatography (GC) was performed on a Shimadzu gas chromatograph (GC-9A; Noisiel, France) (3% OV-1 silanised chromosorb W, column temperature 180 °C, injection port and detector temperature 275–300 °C, flow rate 35 mL/min, flame-ionisation detector). Chromatographic separations were carried out using aluminium sheets pre-coated with silica gel 60 F254 (20 cm × 20 cm, 0.2 mm thick; E. Merck; Darmstadt, Germany) for thin layer chromatography (TLC) and silica gel (230–400 mesh; Darmstadt, Germany) for column chromatography. TLC plates were visualised under UV at 254 and 366 nm and by spraying with ceric sulphate reagent solution (by heating).

3.2. Plant material
The whole plant of *C. oxyacantha* M. Bieb. (05 kg dry weight) was collected from the fields opposite to Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, in May 2012 and was identified by Mr. Muhammad Waris, Plant Taxonomist, Cholistan Institute for Desert Studies, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, where a voucher specimen is deposited (CO-129/12).

3.3. Extraction and isolation
The shade dried whole plant material of *C. oxyacantha* was extracted thrice in methanol (3 L × 20 L) at room temperature and concentrated on rotary evaporator at 35 °C to a dark gummy mass (150 g) which was subjected to silica gel column chromatography and eluted with *n*-hexane, *n*-hexane-dichloromethane (DCM), DCM, DCM-methanol and methanol in increasing order of polarity to get seven fractions (*F*1–*F*7). The fraction *F*1 obtained using *n*-hexane:DCM (8:2) was further purified using *n*-hexane:DCM (7.5:2.5) yielded 1-octacosanol (20 mg). The fraction *F*2 obtained with solvent system *n*-hexane:DCM (7:3) which on further purification using *n*-hexane:DCM (6.5:3.5) resulted in the purification of β-sitosterol (54 mg). The fraction *F*3 obtained using 80% DCM in *n*-hexane, subjected to repeated CC, on elution with 85% DCM in *n*-hexane gave oxyacanthin A (1) (35 mg). The fraction *F*4 obtained DCM:methanol (8.8:0.2) on further purification with 3% methanol in DCM afforded β-sitosterol 3-β-d-glucopyranoside (60 mg). The fraction *F*5 got using 6% DCM:methanol (9.4:0.6) subject on further purification by CC using methanol in DCM afforded luteolin-7-β-glucopyranoside (42 mg). The fraction *F*6 was obtained using 10% methanol in DCM, on further elution with 11% methanol in DCM oxyacanthin B (2) (44 mg).

3.3.1. Oxyacanthin A (1)
Colourless amorphous powder (35 mg); [α]D<sup>24</sup> + 41.0 (c 0.0014 g/mL, MeOH); IR (KBr cm<sup>−1</sup>) ν<sub>max</sub>: 3500, 3450, 2928, 1670, 1650; HR-EI-MS: m/z 709.6567 [M]+ (calcd for C<sub>44</sub>H<sub>87</sub>N<sub>5</sub>O<sub>5</sub>, 709.6584);<sup>1</sup>H NMR data (CDCl<sub>3</sub> + CD<sub>3</sub>OD; 500 MHz), δ: 3.63 (1H, dd, J<sub>1a</sub> = 11.5, 3.8 Hz, H-1a), 3.57 (1H, dd, J<sub>1b</sub> = 11.5, 5.0 Hz, H-1b), 3.91 (1H, dd, J = 4.1, 3.7 Hz, H-2), 3.46 (1H, dd, J = 11.5, 5.0 Hz, H-1b), 3.46 (1H, dd, J = 4.1, 3.7 Hz, H-3), 3.38 (1H, m, H-4), 1.61 (4H, m, H-5′,3′), 1.08 (60H, br s, H-6-25,8′-17′), 0.71 (6H, t, J = 6.5, Hz, H-26,18′), 7.80 (1H, d, J = 8.8 Hz, H-1′), 3.82 (1H, dd, J = 6.6, 3.4, Hz, H-2′), 1.54 (4H, m, H-4′,7′), 5.32 (2H, br m, H-5′,6′).<sup>13</sup>C NMR data (CDCl<sub>3</sub> + CD<sub>3</sub>OD; 125 MHz), δ: 60.6 (C-1), 51.5 (C-2), 75.1 (C-3), 75.1 (C-4), 33.7 (C-5), 29.1–30.2 (C-6-25, 8′-17′), 14.5 (26, 18′), 173.2 (C-1′), 72.9 (C-2′), 35.7 (C-3′), 35.5 (C-4′), 131.4 (C-5′,6′), 33.8 (C-7′).

3.3.2. Oxyacanthin B (2)
Colourless gummy solid (44 mg); [α]<sub>D</sub> + 33.5 (c 0.0012 g/mL, MeOH); IR (KBr cm<sup>−1</sup>) ν<sub>max</sub>: 3505, 3440, 2919, 1670, 1655; HR-FAB-MS (-ve mode) m/z 870.7021 [M–H]<sup>+</sup> (calcd for C<sub>50</sub>H<sub>96</sub>N<sub>10</sub>O<sub>10</sub>)
870.7034). 1H NMR data (CD3OD; 500 MHz), δ: 4.04 (1H, dd, J = 11.6, 3.9 Hz, H-1a), 3.87 (1H, dd, J = 11.6, 5.1 Hz, H-1b), 3.81 (1H, m, H-2), 4.24 (1H, m, H-3), 3.60 (1H, m, H-4), 1.61 (4H, m, H-5, 3′), 1.08 (60H, br s, H-6-25, 8′–17′), 0.71 (6H, t, J = 6.4 Hz, H-6′, 18′), 7.82 (1H, d, J = 8.7 Hz, N–H), 4.06 (1H, dd, J = 6.4, 3.6 Hz, H-2′), 1.54 (4H, m, H-4′), 5.41 (2H, br m, H-5′, 6′), 4.28 (1H, d, J = 7.5 Hz, H-1″), 3.39 (1H, m, H-3″), 3.36 (1H, m, H-2″), 3.65 (1H, m, H-4″), 3.79 (1H, m, H-5″), 3.85 (1H, dd, J = 10.4, 2.9 Hz, H-6″a), 3.71 (1H, dd, J = 10.4, 2.9 Hz, H-6″b).

13C NMR data (CD3OD; 125 MHz), δ: 69.9 (C-1), 51.6 (C-2), 75.5 (C-3), 73.0 (C-4), 33.7 (C-5), 29.1–30.2 (C-6-25, 8′–17′), 14.5 (C-26, 18′), 177.1 (C-1′), 72.9 (C-2′), 35.7 (C-3′), 35.5 (C-4′), 131.4 (C-5′, 6′), 33.8 (C-7′), 104.7 (C-1″), 77.9 (C-2″), 72.9 (C-3″), 71.5 (C-4″), 78.0 (C-5″), 62.6 (C-6″).

3.4. Methanolysis
Compounds 1 and 2 (10 mg each) were refluxed separately with 5 mL of 1 N HCl and 20 mL of MeOH for 15 h. The reaction mixture was then extracted with n-hexane to obtain the corresponding fatty acid methyl esters, which were analysed by GC–MS after acetylation with acetic anhydride-pyridine. The aqueous layer of 2 was evaporated to dryness, and the residue was separated by silica gel column chromatography as sphingosine base and methylated sugar. The base was acetylated and analysed by GC–MS. The sugar was identified as methyl β-d-glucopyranoside based on the sign of optical rotation [α]D + 77.1 (c 0.001, MeOH) and Co-TLC profile (Rf 0.45 (EtOAc/MeOH/H2O; 5:2:0.5)).

3.4.1. Methyl ester derived from 1
[α]D24 + 8.1 (c 0.001); 1H NMR (CDCl3; 400 MHz), δ: 5.31 (2H, d, J = 15.2, 5.4, H-5′, 6′), 4.11 (1H, t, J = 6.4, H-2′), 3.51 (3H, s, MeO), 2.13 (4H, m, H-4′, 7′), 1.98 (3H, s, MeCO), 1.19–1.29 (20H, br s, CH2-8′–17′), 0.85 (3H, t, J = 6.5, CH3-18′); GC–MS: m/z 354.

3.4.2. Methyl ester derived from 2
[α]D24 + 11.2 (c 0.0011); 1H NMR (CDCl3; 400 MHz), δ: 5.30 (2H, d, J = 16.0, 5.5, H-5′, 6′), 4.17 (1H, t, J = 6.5, H-2′), 3.54 (3H, s, MeO), 2.12 (4H, m, H-4′, 7′), 2.00 (3H, s, MeCO), 1.20–1.31 (20H, br s, CH2-8′–17′), 0.87 (3H, t, J = 6.7, CH3-18′); GC–MS: m/z 354.

3.4.3. Acetylsphingamine derived from 1
[α]D24 + 18.9 (c 0.0014); 1H NMR (CDCl3; 400 MHz), δ: 7.92 (1H, d, J = 7.7, NH), 4.64 (1H, dd, J = 5.1, 4.0, H-4), 4.54 (1H, m, H-2), 4.43 (1H, dd, J = 10.1, 5.4, H-1), 4.33 (1H, dd, J = 10.1, 3.1, H-1), 4.19 (1H, dd, J = 5.1, 3.1, H-3), 2.00 (12H, 4 × MeCO), 1.16–1.27 (40H, br s, CH2-6–25), 0.86 (t, J = 6.5, CH3-26); GC–MS: m/z 597.

3.4.4. Acetylsphingamine derived from 2
[α]D24 + 19.6 (c 0.012); 1H NMR (CDCl3; 400 MHz), δ: 7.91 (1H, d, J = 8.0, NH), 4.61 (1H, dd, J = 5.0, 4.1, H-4), 4.52 (1H, m, H-2), 4.40 (1H, dd, J = 10.6, 5.2, H-1), 4.30 (1H, dd, J = 10.6, 3.0, H-1), 4.16 (1H, dd, J = 5.0, 3.2, H-3), 2.01 (12H, 4 × MeCO), 1.16–1.26 (40H, br s, CH2-6–25), 0.85 (t, J = 6.5, CH3-26); GC–MS: m/z 597.

3.5. Oxidative cleavage of double bond in 1 and 2
To the fatty acid methyl esters of compounds 1 and 2 (4 mg each) in acetone, added 1 mL of 0.04 M solution of K2CO3, 6 mL of an aqueous solution 0.025 M KMnO4 and 0.09 M NaIO4 in 100 mL round-bottom flask, separately. The reaction was allowed to proceed at 37 °C for 18 h. After acidification with 5 N H2SO4, the solution was decolorised with a 1 M solution of
oxalic acid and extracted with Et₂O (3–10 mL). The combined organic extracts were dried over Na₂SO₄, filtered and concentrated. The resulting carboxylic acids were methylated with ethereal solution of diazomethane, analysed by GC–MS and found methyl 2-methoxypenta-1,5-dioate (m/z 190) and methyl tridecanoate (m/z 228), respectively.

3.6. Lipoxygenase inhibition assay
The LOX activity was assayed according to the method (Tappel 1953) with slight modifications. A total volume of 200 µL assay mixture contained 160 µL sodium phosphate buffer (100 mM, pH 8.0), 10 µL (0.5 mM) of test compound and 20 µL purified lipoxygenase (EC 1.13.11.12) type-I-B from Glycine max (600 units per well, Sigma-Aldrich Chem. Co., St. Louis MO, USA. Cat. No. 9029-60-1). The contents were pre-incubated for 6 min at 25 °C. The reaction was initiated by addition of 10 µL linoleic acid (substrate solution). The change in absorbance was observed after 6 min at 234 nm. All reactions were performed in triplicates in 96-well microplate reader (Synergy HT, BioTek, USA). The positive and negative controls were included in the assay. The percentage inhibition (%) was calculated by formula:

\[
\text{Inhibition (\%)} = \left( \frac{\text{Control} - \text{Test}}{\text{Control}} \right) \times 100
\]

Control is the total enzyme activity without inhibitor and test is the activity of the test compound. IC₅₀ values (concentration at which there is 50% enzyme inhibition) were determined after suitable dilutions of the compounds and results computed using EZ-Fit Enzyme kinetics software (Perrella Scientific Inc., Amherst, USA).

3.7. Cholinesterase inhibition assay
Electric eel (Electrophorus electricus) AChE (EC 3.1.1.7, type VI-S), equine-serum BChE (EC 3.1.1.8) inhibiting activities were measured by the method (Ellman et al. 1961). All chemicals were purchased from Sigma-Aldrich Co., USA. Total volume of the reaction mixture 100 µL contained 60 µL KH₂PO₄ buffer (100 mM, pH 7.7). Ten µL test compound (0.5 mM per well) was added, followed by the addition of 10 µL enzyme (0.05 units AChE & 0.5 units BChE). The contents were mixed, pre-incubated for 10 min at 37 °C and pre-read at 405 nm. The reaction was initiated by the addition of 10 µL of 0.5 mM per well of respective substrate, followed by the addition of 10 µL DTNB (0.5 mM per well). After 15 min, absorbance was measured at 405 nm. Data were computed as described above for lipoxygenase assay.

4. Conclusion
Seven compounds were isolated from the whole plant material of C. oxyacantha, including two new sphingolipids named as oxyacanthins A, B (1, 2). Both the compounds 1 and 2 showed inhibitory potential against enzyme lipoxygenase with IC₅₀ values 83.3 ± 1.3 and 245.7 ± 1.1 µM. However, compound 2 showed inhibitions against enzymes AChE and BChE with IC₅₀ values 65.3 ± 0.1 and 93.6 ± 0.1 µM, respectively.

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