Structural Elucidation and Toxicity Evaluation of Bioactive Compounds from the Leaves and Stem woods of *Synadenium glaucescens* Pax.

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ABSTRACT

The leaves and stem woods of *Synadenium glaucescens* Pax. are traditionally used for treatment of various human diseases in Tanzania. However, there is limited information on the structural elucidation and toxicity properties of bioactive compounds responsible for their pharmacological properties. Thus, this study was aimed to elucidate and evaluate toxicity properties of bioactive compounds from these parts. Separation of bioactive compounds was achieved by using column chromatographic method, whereas their structures were determined by GC-MS and NMR data and by comparing with literature spectroscopic data. Toxicity properties of isolated compounds was done by brine shrimp lethality test (BSLT) method. In this study, column chromatographic separation led to isolation of tetracosane (1), hexadecyl palmitate (2), octacosane (3), tetracosanol (4) and β-sitosterol (5) from the leaves and 1-hexacosene (6), campesterol (7), octadecanoic acid (8) and tetracosanoic acid (9) from the stem woods. BSLT of compounds isolated in sufficient amount (1, 2, 3, 4, 5 and 7) were revealed to be non-toxic to brine shrimp larvae (LC_{50} >100 µg/ml). Except compound 5, this study reports the isolation of other eight (8) compounds for the first time from this plant. Therefore, it can be reasonably concluded that *S. glaucescens* is a good source of bioactive compounds which justify its traditional uses in treatment of various ailments. Additionally, observed non-toxic effect of the tested compounds indicates safety and it is expected that its traditional use has no toxicity effect.

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INTRODUCTION

In Tanzania, *Synadenium glaucescens* Pax (family Euphorbiaceae) is one of the useful medicinal plants traditionally used for treatment of various diseases both in humans and animals (Mabiki et al*.,* 2013a). For instance, in Njombe region-Southern Highlands of Tanzania, its leaves and stem woods are traditionally used for treatment of human immunodeficiency virus (HIV), cough or tuberculosis (TB), asthma, leprosy, sores, wounds and worms in humans and healing of east coast fever (ECF) in cattle and Newcastle disease in poultry (Mabiki et al*.,* 2013; Nyigo et al*.,* 2016; Nyigo et al*.,* 2015). Previous pharmacological studies have demonstrated that, several parts of this plant including the leaves and stem woods have antimicrobial activities (Mabiki et al*.,* 2013a, 2013b; Max et al*.,* 2014).

Despite the vast traditional uses of this plant, both externally and internally, in the treatment of both humans and animals diseases, it has been previously implicated for causing mild to moderate dermal irritancy (Nyigo et al*.,* 2015).

Pharmacological and toxicological properties of medicinal plants, including *S. glaucescens,* are attributed by the presence of phytochemical compounds they produce (Faridha et al*.,* 2016). Previous phytochemical screening of crude extracts from various parts of *S. glaucescens* revealed to possess several classes of phytochemical compounds such as terpenoids, steroids, tannins, coumarins, glycosides and phenolics (Mabiki et al*.,* 2013).

Despite the possession of many phytochemical compounds and its usefulness in traditional medicine as described above, information about structural elucidation/identification and toxicity evaluation of its phytochemical compounds are limited.

The only available information is the identification of β-sitosterol (5) and euphol (10) from its root barks and leaves respectively (Nyigo et al*.,* 2016), erythrinacinate C (11) and 1-octacosanol (12) from its root barks and leaves respectively (Nyigo et al*.,* 2022), and 3β-friedelanol (13) and 3α-friedelanol (14) from its leaves (Credo et al*.,* 2022).

Therefore, this study was undertaken to further identify and evaluate toxicity of bioactive compounds from the leaves and stem woods of *S. glaucescens*.

METHODS

General
All chemicals used in this study were of analytical grade. They were obtained from either Loba Chemie, Mumbai-India, i.e. dichloromethane (DCM) and petroleum ether (PE), or Finar Chemical, Gujarat-India, i.e. methanol (MeOH). The silica gel 60 (70-230 mesh, 60 angstrom pore size) and precoated thin layer chromatography aluminium sheets (TLC, silica gel 60 F_{254}) were obtained from Merck KGaA group, Darmstadt, Germany. The brine shrimps (*Artemia franciscana*) eggs and sea salt were obtained in the Department of Chemistry and Physics, Sokoine University of Agriculture-Tanzania.

Identification, Collection and Preparation of Plant Materials
Identification of plant species was done by a botanist from the Department of Botany, University of Dar es Salaam (UDSM). Its leaves and stem woods were collected from Njombe district (08°34′ to 08°49′ S and 034°55′ to 035°10′ E), Njombe region, Tanzania in December 2018 and the voucher no. 3672 was stored in the herbarium of the Department of Botany- UDSM. Plant materials were air dried in an open space under shade at room temperature, then pulverized into fine powder by using a milling machine type Y (Hangyu®, China).

Extraction of Plant Materials
1 kg of each powdered part of *S. glaucescens* was extracted exhaustively with 100% dichloromethane (DCM) by soxhlet method (Azwanida, 2015). The filtrates obtained were evaporated using a rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) at 40°C to obtain DCM extracts. The residues of each plant part obtained was dried and further exhaustively extracted with 100% MeOH by maceration method. The filtrates obtained were evaporated to obtain MeOH extracts.

Isolation and Purification of Bioactive Compounds
40 g of 100% DCM extract from the leaves was chromatographed on silica gel by gradient elution using PE, DCM and MeOH. By using TLC to monitor separations, the column was eluted by solvent containing 25% DCM in PE to obtain 30 fractions (Fr. 1-30), 50 ml each. Solvent polarity was further increased to 50% of DCM in PE to give 50 fractions (Fr. 31-80). Furthermore, the solvent polarity was increased to 75% of DCM in PE to yield 63 fractions (Fr. 81-143) followed by 100% DCM to give 8 fractions (Fr. 144-151). Then, solvent polarity was increased to 10% of MeOH in DCM giving

14 fractions (Fr. 152-165), finally increased to 20% of MeOH in DCM affording 5 fractions (Fr. 166-170).

On TLC analysis, twelve fraction combinations with similar chemical profiles, i.e. Fr. 1-12 Fr. 13-21, Fr. 22-30, Fr. 31-38 Fr. 39-65, Fr. 66-74, Fr. 75-80, Fr. 81-101, Fr. 102-143, Fr. 144-151, Fr. 152-165 and Fr. 166-170. Upon settling at room temperature, the first (Fr. 1-12), second (Fr. 13-21), fifth (Fr. 39-65) and eleventh (Fr. 152-165) fraction combinations were observed to form precipitates. The precipitates were filtered by washing with 100% MeOH and dried to obtain pure compound 1, 2, 3 and 4 respectively.

Similarly, 40 g of 100% MeOH extract from the leaves was also chromatographed on silica gel by gradient elution using PE, DCM and MeOH. The column was eluted by 25% of DCM in PE to afford 14 fractions (Fr. 1-14), and then increased to 50% of DCM in PE to give 3 fractions (Fr. 15-17). Furthermore, polarity was increased to 75% of DCM in PE to yield 17 fractions (Fr. 18-34) followed by 100% DCM to obtain 25 fractions (Fr. 35-59). Then, solvent polarity was further increased to 10% of MeOH in DCM to give 5 fractions (Fr. 60-64) and finally to 20% of MeOH in DCM to give 15 fractions (Fr. 65-79).

On TLC analysis, eight fraction combinations with similar chemical profiles (Fr. 1-14, Fr. 15-17, Fr. 18-34, Fr. 35-59, Fr. 60-61, Fr. 62, Fr. 63-64 and Fr. 65-79) were obtained. In the fifth (F60-61) fraction combination, precipitates were observed to develop when settled at room temperature. The formed were filtered by washing with 100% MeOH and dried to obtain pure compound 5.

On the other hand, 20 g of 100% DCM extract from the stem woods was chromatographed on silica gel by gradient elution with PE, DCM and MeOH. Column was eluted with 25% of DCM in PE to afford 20 fractions (Fr. 1-20), then to 50% of DCM in PE to give 16 fractions (Fr. 21-36). Solvent polarity was further increased to 75% of DCM in PE to afford 10 fractions (Fr. 37-46) followed by 100% DCM to give 13 fractions (Fr. 47-59). Then increased to 10% of MeOH in DCM to give 10 fractions (Fr. 60-69) and finally to 20% of MeOH in DCM to give 9 fractions (Fr. 70-78).

Nine fraction combinations with similar chemical profiles (Fr. 1-3, Fr. 4-20, Fr. 21-36, Fr. 37-39, Fr. 40-46, Fr. 47-53, Fr. 54-59, Fr. 60-69 and Fr. 70-78) were obtained after TLC analysis. The first (Fr. 1-3), fourth (Fr. 21-36) and sixth (Fr. 47-53) fraction combinations were observed to form precipitates when settled at room temperature. The precipitates were filtered by washing with 100% MeOH and dried to obtain pure compound 6, 7 and 8 respectively.

Finally, 50 g of 100% MeOH extract from the stem woods was chromatographed on silica gel by gradient elution with PE, DCM and MeOH. Column was eluted with 25% of DCM in PE to give 14 fractions (Fr. 1-14) and then increased to 50% of DCM in PE to give10 fractions (Fr. 15-24). Furthermore, solvent polarity was increased to 75% of DCM in PE to obtain 19 fractions (Fr. 25-43) followed by 100% DCM to afford 7 fractions (Fr. 44-50) and finally to 10% of MeOH in DCM to give 14 fractions (Fr. 51-64).

TLC analysis led to form seven fraction combinations with similar chemical profiles (Fr. 1-12, Fr. 13-14, Fr. 15-24, Fr. 25-43, Fr. 44-45, Fr. 46-50 and Fr. 51-64). The second (Fr. 13-14) fraction combination was observed to develop precipitates after settling at room temperature. The precipitates were then filtered by washing with 100% MeOH and dried obtain pure compound 9.

All fraction combinations which did not form precipitates were few in amount (less than 0.5 mg), hence, not subjected to further purification.

Spectroscopic Analysis
Mass spectrum having molecular ion peak, $[M]^+$ of each compound was recorded in electron impact (EI) mode by an Agilent 5973 Triple Quadrupole GC-MS system (from California, USA) with the National Institute of Standards and Technology (NIST) spectral library (version 2002). Based on the value of mass to charge (m/z) ratio of $[M]^+$, the structure of each compound was suggested by the NIST spectral library.

The ^1H and ^{13}C NMR data of isolated compounds were recorded on a 600 Megahertz Bruker Avance III HD 600 (UltraShield™) NMR spectrometer (from Massachusetts, USA) with tetramethylsilane (TMS) as internal standard. The acquired data were processed by using Topspin software version 3.6.3.

Brine Shrimp Lethality Test (BSLT)
With some minor modifications, toxicity study of compound 1, 2, 3, 4, 5 and 7 was done by BSLT method according to previous described procedure (Magadula et al*.*, 2012). In brief, 40 mg/ml stock solution of each compound dissolved in 20% dimethylsulphoxide (DMSO) were made. Five concentration levels (1000, 800, 600, 400 and 200 µg/mL) were made by drawing different volumes from the stock solutions and then added into vials, each having ten brine shrimp larvae. Then, volume of vial was adjusted to 5 mL with artificial seawater prepared by dissolving 3.8 g of sea salt in 1 L of distilled water. Each concentration was tested in duplicate. The negative control containing artificial seawater, brine shrimp and 20% DMSO was prepared. The vials were then incubated under light for

24 h. The number of died brine shrimps for each vial after incubation were counted and their mean at each concentration was determined.

Then, mean percentage of mortality at each concentration was determined using the equation:

$$Percentage\ Mortality = \frac{Number\ of\ dead\ nauplii}{Number\ of\ nauplii\ added\ in\ a\ vial}x\ 100 \quad (Waghulde\ et\ al.,2019) \quad \textbf{(1)}$$

The mean percentage mortality was subjected to regression analysis using Microsoft Excel (version 2016). The mean percentage mortality was plotted against the logarithm of concentrations. The regression equations obtained from the graphs were used to determine the lethal concentrations for 50% mortality of the larvae (LC_{50}) and the 95% confidence interval (95% CI) values as per previous described procedure (Magadula et al*.,* 2012; Nondo et al*.,* 2015). The results of brine shrimp toxicity were interpreted as follows: $LC_{50} < 1.0$ µg/ml as highly toxic; LC_{50} 1.0-10.0 µg/ml as toxic; LC_{50} 10.0-30.0 µg/ml as moderately toxic; LC_{50} >30-100 ml as mildly toxic and $LC_{50} > 100$ µg/ml as non-toxic (Nondo et al*.,* 2015).

RESULTS AND DISCUSSION

Isolation of Bioactive Compounds
Compounds 1-4 were isolated from 100% DCM leaves extract of *S. glaucescens*; while compound 1 was white crystals with 1729 mg, and compound 2 was colorless waxy with 1249 mg. Compound 3 and 4 were white amorphous powder with 260 and 91 mg respectively. Compound 5 was isolated from MeOH leaves extract as white amorphous powder with 84 mg.

Moreover, compounds 6-8 were isolated from DCM stem woods extract; compound 6 was white waxy with 12 mg while compound 7 and 8 were white amorphous powder with 88 and 25 mg respectively. Finally, compound 9 was isolated from MeOH stem woods extract as white amorphous powder with 23 mg. All isolated compounds showed single spot on TLC analysis to confirm their purity.

Structure Determination
Based on experimental MS and NMR data and comparison with other published spectroscopic data, chemical structures of compounds 1-9 were determined as follows:

Compound 1: EI-MS m/z 338 $[M]^+$ ($C_{24}H_{50}$) corresponding to tetracosane (1) as suggested by NIST library. ^1H NMR (600 MHz, $CDCl_3$): δ (ppm) 0.86 (-CH_3, *t*, *J* =7.1 Hz) 1.23 (-CH_2, *s*) and 1.52 (-CH_2, *s*). ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 14.34 (CH_3), 22.92 (CH_2), 29.59 (CH_2), 29.92 (CH_2) and 32.15 (CH_2). The

above experimental spectral data are in consistent with the literature data (Umaru et al., 2019).

Compound 2: EI-MS *m/z* 480 [M]$^+$ (C$_{32}$H$_{64}$O$_2$) corresponding to hexadecyl palmitate (2) as suggested by NIST library. It is also known as cetyl palmitate (hexadecyl hexadecanoate or palmityl palmitate). ^{1}H NMR (600 MHz, d-CD$_2$Cl$_2$): δ (ppm) 0.88 (-CH$_3$, *t*, *J* = 6.9 Hz), 1.26 (-CH$_2$, *s*), 1.52 (-CH$_2$, *s*), 1.61 (-CH$_2$, *m*), 2.26 (-CH$_2$, *t*, *J* = 7.5 Hz) and 4.02 (-CH$_2$, *t*, *J* = 6.7 Hz). ^{13}C NMR (150 MHz, d-CD$_2$Cl$_2$): δ (ppm) 174.23 (-C=O), 64.80 (-CH$_2$-O), 34.89 (-CH$_2$-C=O), 32.52, 30.27, 29.94, 29.27, 26.53, 25.62, 23.28 (each, –CH$_2$) and 14.45 (-CH$_3$). The experimental spectral data above are in consistent with the literature data (Mir et al., 2020).

Compound 3: EI-MS *m/z* 394 [M]$^+$ (C$_{28}$H$_{58}$) corresponding to octacosane (3) as suggested by NIST library. ^{1}H NMR (600 MHz, d-CD$_2$Cl$_2$): δ (ppm) 0.88 (-CH$_3$, *t*, *J* =7.1 Hz), 1.26 (-CH$_2$, *s*) and 152 (-CH$_2$, *s*). ^{13}C NMR (150 MHz, d-CD$_2$Cl$_2$): δ (ppm) 14.44 (CH$_3$), 23.27 (CH$_2$) 29.94 (CH$_2$), 30.26 (CH$_2$) and 32.51 (CH$_2$). The experimental spectral data are in consistent with the literature data (Molo et al., 2021; Tesemma et al., 2013) chloroform, acetone, methanol and water.

Compound 4: EI-MS *m/z* 354 [M]$^+$ (C$_{24}$H$_{50}$O) corresponding to tetracosanol (4) as suggested by NIST library. ^{1}H NMR (600 MHz, CDCl$_3$): δ (ppm) 0.88 (-CH$_3$, *t*, *J* = 6.9 Hz), 1.26 (-CH$_2$, *m*) and 3.58 (2H, *t*, *J* = 6.7 Hz, -CH$_2$OH). ^{13}C NMR (150 MHz, CDCl$_3$): δ (ppm) 14.45 (-CH$_3$), 23.27, 26.35, 29.94, 30.04, 30.27, 32.51, 33.49 (each, –CH$_2$) and 63.42 (-CH$_2$OH). The experimental spectral data above are in consistent with the literature data (Makhafola et al., 2017; Nandini & Vaidya, 2019).

Compound 5: EI-MS *m/z* 414 [M]$^+$ (C$_{29}$H$_{50}$O) corresponding to β-sitosterol (5) as suggested by NIST library. ^{1}H NMR (600 MHz, CDCl$_3$): two methyl singlets at δ (ppm) 0.66 (3H, *s*) and 0.99 (3H, *s*); three methyl doublets at δ 0.80 (3H, *d*, *J* = 6.4 Hz), 0.81 (3H, *d*, *J* = 6.4 Hz) and 0.90 (3H, *d*, *J* = 6.5 Hz); one methyl triplet at δ 0.82 (3H, *t*, *J* = 7.2 Hz); proton connected to C-3 hydroxyl group at 3.50 (1H, *tdd*, *J* = 4.5, 4.2, 3.8 Hz) and one olefinic proton at δ 5.33 (1H, *t*, *J* = 6.4 Hz). ^{13}C NMR (150 MHz, CDCl$_3$): δ (ppm) 37.5 (C-1), 31.9 (C-2), 72.0 (C-3), 42.5 (C-4), 140.9 (C-5), 121.9 (C-6), 32.1 (C-7), 32.1 (C-8), 50.4 (C-9), 36.7 (C-10), 21.3 (C-11), 39.9 (C-12), 42.5 (C-13), 56.9 (C-14), 24.5 (C-15), 28.5 (C-16), 56.3 (C-17), 36.4 (C-18), 19.3 (C-19), 34.2 (C-20), 26.3 (C-21), 46.1 (C-22), 23.3 (C-23), 12.2 (C-24), 29.4 (C-25), 20.0 (C-26), 19.6 (C-27), 18.9 (C-28) and 12.1 (C-29). The experimental spectral data above are in consistent with the literature data (Chaturvedula & Prakash, 2012).

Compound 6: EI-MS *m/z* 364 [M]$^+$ (C$_{26}$H$_{52}$) corresponding to 1-hexacosene (6) as suggested by NIST library. ^{1}H NMR (600 MHz, CDCl$_3$): one methyl singlet at δ (ppm) 0.86 (-CH$_3$, *t*, *J* =3.12 HZ), broad signals at δ 1.23-1.36 (CH$_2$, *br*), signal of methylene protons next to olefinic methine proton at δ 2.03 (-CH$_2$, *m*), doublet of doublets signals of terminal olefinic methylene protons at δ 4.97 (1H, *dd*, *J* =17.19, 1.4 Hz) and 4.91 (1H, *dd*, *J* =10.18, 1.4 Hz) and a broad signal of olefinic methine proton at δ 5.80 (1H, *m*). ^{13}C NMR (150 MHz, CDCl$_3$): signal of olefinic terminal methylene carbon at δ (ppm) 114.3, signal of olefinic methine carbon at δ 139.5, signals of a cluster of methylene carbons at δ 22.9-34.1 and a signal of methyl carbon at δ 14.3. The experimental spectral data above are in consistent with the literature data (Lavilla et al., 2014).

Compound 7: EI-MS *m/z* 400 [M]$^+$ (C$_{28}$H$_{48}$O) corresponding to campesterol (7) as suggested by NIST library. ^{1}H NMR (600 MHz, CDCl$_3$): two methyl singlets at δ (ppm) 0.66 (-CH$_3$, *s*) and 0.99 (-CH$_3$, *s*); four methyl doublets at δ 0.80 (-CH$_3$, *d*, *J* = 7.0 Hz), 0.81 (-CH$_3$, *d*, *J* = 7.2 Hz) and 0.90 (-CH$_3$, *d*, *J* = 6.6 Hz) and 0.82 (-CH$_3$, *d*, *J* = 7.2 Hz); proton connected to C-3 hydroxyl group at 3.50 (1H, *m*) and one olefinic proton at δ 5.33 (1H, *brd*, *J* = 5.2 Hz). ^{13}C NMR (150 MHz, CDCl$_3$): δ (ppm) 37.9 (C-1), 28.8 (C-2), 72.3 (C-3), 42.9 (C-4), 141.6 (C-5), 122.0 (C-6), 32.5 (C-7), 29.8 (C-8), 50.8 (C-9), 37.1 (C-10), 19.8 (C-11), 40.4 (C-12), 42.9 (C-13), 57.4 (C-14), 23.6 (C-15), 26.6 (C-16), 56.7 (C-17), 20.1 (C-18), 12.2 (C-19), 32.3 (C-20), 19.4 (C-21), 34.5 (C-22), 21.7 (C-23), 46.5 (C-24), 36.7 (C-25), 18.9 (C-26), 19.1 (C-27) and 24.8 (C-28). The experimental spectral data above are in consistent with the literature data (Musa et al., 2019).

Compound 8: EI-MS *m/z* 284 [M]$^+$ (C$_{18}$H$_{36}$O$_2$) corresponding to octadecanoic acid (8) as suggested by NIST library. It is also known as stearic acid. ^{1}H NMR (600 MHz, CDCl$_3$): δ 0.86 (-CH$_3$, *t*, *J* = 6.6 Hz), 1.23 (-CH$_2$, *s*), 1.61 (-CH$_2$, *s*), 2.32 (-CH$_2$, *t*, *J* = 7.3 Hz) and 8.10 (OH, *s*). ^{13}C NMR (150 MHz, CDCl$_3$): δ (ppm) 178.92 (-C=O), 34.05 (-CH$_2$-C=O), 32.14, 29.92, 29.81, 29.66, 29.58, 29.46, 29.29, 24.92, 22.91 (each, -CH$_2$) and 14.33 (-CH$_3$). The experimental spectral data above are in consistent with the literature data (Abdurrahman & Cai-Xia, 2020).

Compound 9: EI-MS *m/z* 368 [M]$^+$ (C$_{24}$H$_{48}$O$_2$) corresponding to tetracosanoic acid (9) as suggested by NIST library. It is also known as lignoceric acid. ^{1}H NMR (600 MHz, CDCl$_3$): δ (ppm) 0.86 (CH$_3$, *t*, *J* = 6.5 Hz), 1.23 (-CH$_2$, *s*), 1.54 (-CH$_2$, *s*), 2.29 (-CH$_2$, *br*) and 8.08 (OH, *s*). ^{13}C NMR (150 MHz, CDCl$_3$): δ (ppm) 165.52 (-C=O), 34.0 (-CH$_2$-C=O), 33.05, 32.15, 29.93, 29.59,
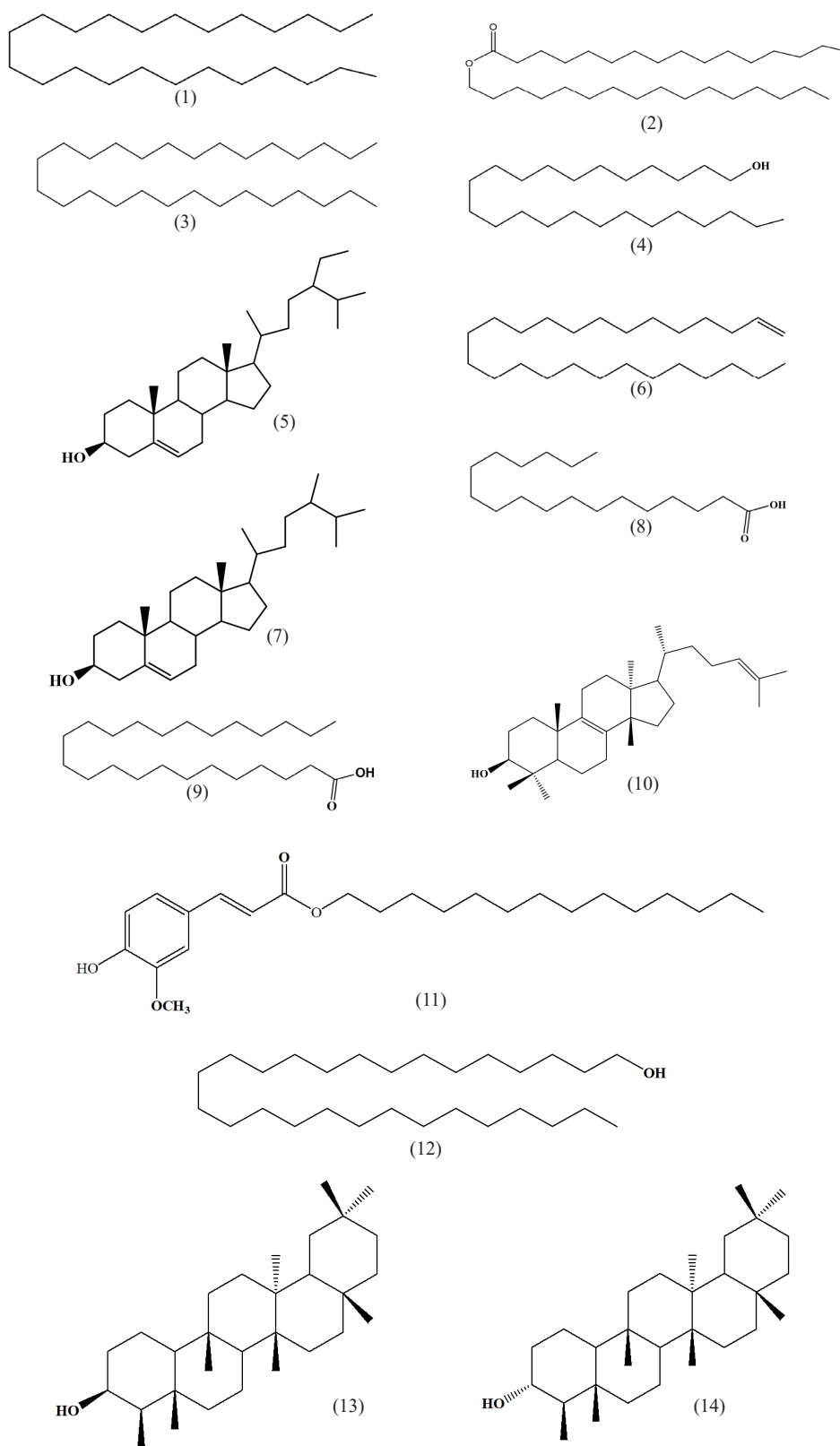
Figure 1. Structures of compounds 1-14

Table 1. Brine shrimp toxicity of tested compounds

Compound name	LC_{50} (µg/ml)	95% Confidence interval (µg/ml)	
		Lower limit	Upper limit
Tetracosane **(1)**	1.03×10^4	-124.39	40.03
Hexadecyl palmitate **(2)**	1.18×10^4	-124.41	41.32
Octacosane **(3)**	2.65×10^5	-211.65	51.58
Tetracosanol **(4)**	2.94×10^5	-211.72	51.61
β-sitosterol **(5)**	1.24×10^4	-124.44	40.23
Campesterol **(7)**	2.73×10^5	-211.69	51.57

25.97, 25.10, 22.92 (each, $-CH_2$) and 14.34 ($-CH_3$). The experimental spectral data above are in consistent with the literature data (Awaad et al*.,* 2016).

Previously, only β-sitosterol (5) and euphol (10) have been isolated from *S. glaucescens* root barks and leaves of respectively (Nyigo et al*.,* 2016), erythrinacinate C (11) and 1-octacosanol (12) from its root barks and leaves respectively (Nyigo et al*.,* 2022) and 3β-friedelanol (13) and 3α-friedelanol (14) from its leaves (Credo et al*.,* 2022).

This study has afforded the isolation of tetracosane (1), hexadecyl palmitate (2), octacosane (3), tetracosanol (4) and β-sitosterol (5) from the its leaves and 1-hexacosene (6), campesterol (7), octadecanoic acid (8) and tetracosanoic acid (9) from its stem woods. Therefore, this study reports the isolation of compound 1, 2, 3, 4, 6, 7, 8 and 9 for the first time from this plant.

Literature search revealed that these compounds are not new since they have been previous isolated from other plant species. Additionally, some of them have been revealed to possess various pharmacological activities. For instance, tetracosane (1) inhibits β-amyloid peptides aggregation in Alzheimer's disease (Lomarat et al*.,* 2015), octacosane (3) inhibits of urease activity (Molo et al*.,* 2021), tetracosanol (4) has anti-proliferative effect (Makhafola et al*.,* 2017), β-sitosterol (5) has anti-benign prostatic hyperplasia, anti-inflammatory, anti-tuberculosis, anti-asthma, anti-cholesterol, diuretic and anti-arthritic (Ragasa et al*.,* 2017; Saeidnia et al*.,* 2014), campesterol (7) exhibits anti-angiogenic activity and anti-cholesterol (Saeidnia et al*.,* 2014) and tetracosanoic acid (9) possesses anti-ulcerogenic activity (Awaad et al*.,* 2016). Therefore, these compounds among others contribute to the reported and observed pharmacological properties of *S. glaucescens*.

Brine Shrimp Lethality Test
BSLT assay was done to predict toxicity properties of bioactive compounds from the leaves and stem woods

of *S. glaucescens*. The results revealed that all tested compounds were non-toxic to brine shrimp larvae (LC_{50} > 100 µg/ml) as shown in Table 1.

BSLT is a convenient method for screening bioactive natural products. Additionally, it is a rapid, cheap and simple bioassay for testing plant extracts bioactivity, which greatly correlates with cytotoxic and antitumor properties (Lavilla et al., 2014). In this study, compound 6, 8 and 9 were not assessed for toxicity since they were obtained in inadequate amount to perform the test.

CONCLUSION

In conclusion, this study demonstrates that *S. glaucescens* is a good source of bioactive compounds, which is a justification for its traditional uses in treatment of several diseases. Furthermore, observed non-toxic effect of its tested compounds indicates safety and perhaps its traditional use has no toxicity effect. However, further studies on toxicity properties of these compounds are recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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Compound 1
1H NMR (600 MHz, CDCl3)

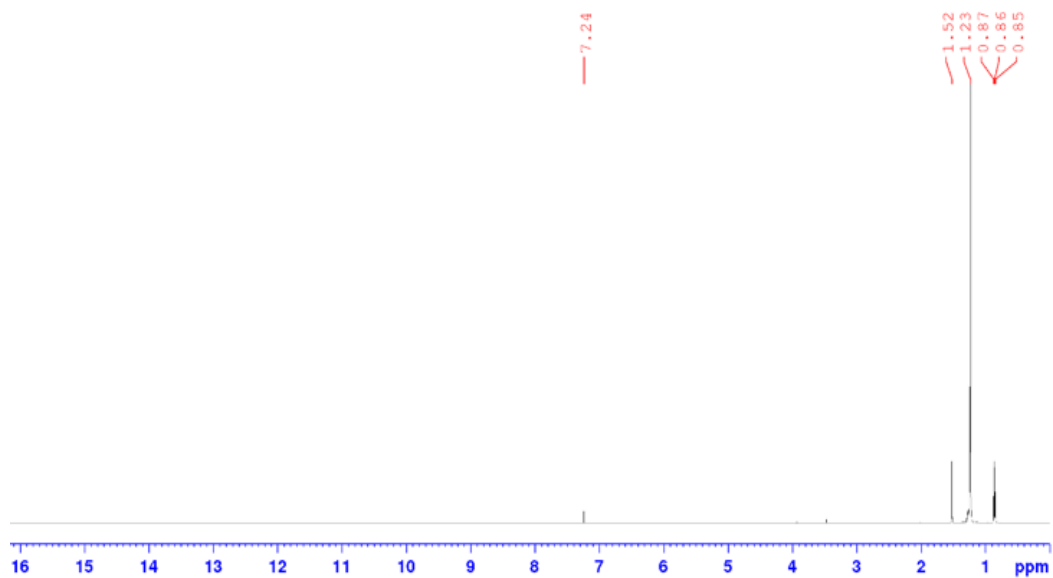**Figure S1. ^{1}H-NMR (600 MHz, CDCl$_3$) spectrum of compound 1 (Tetracosane)**
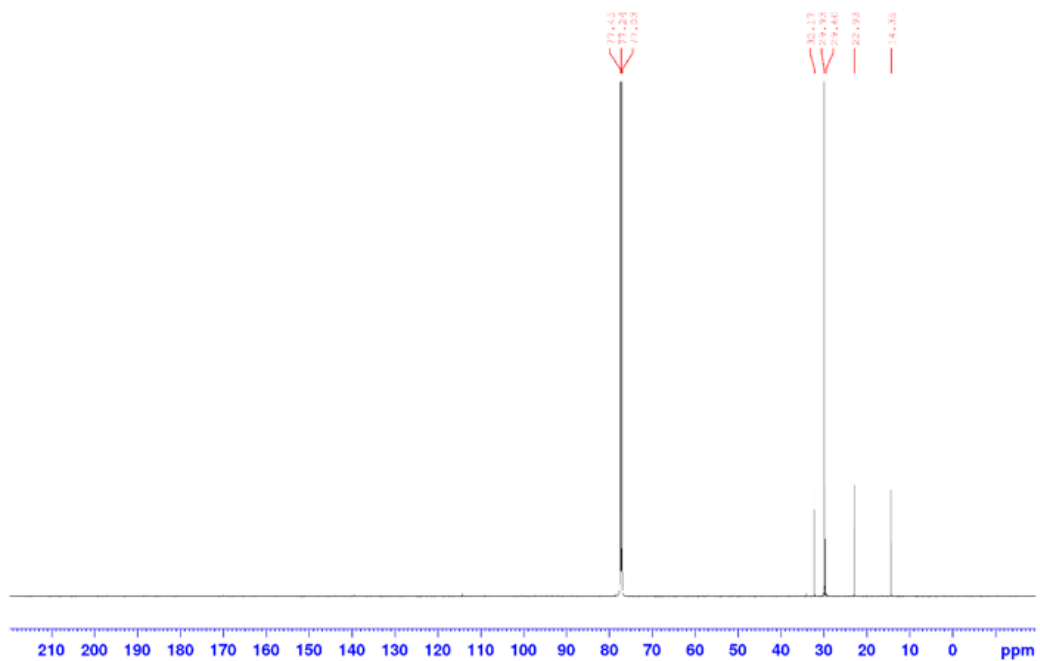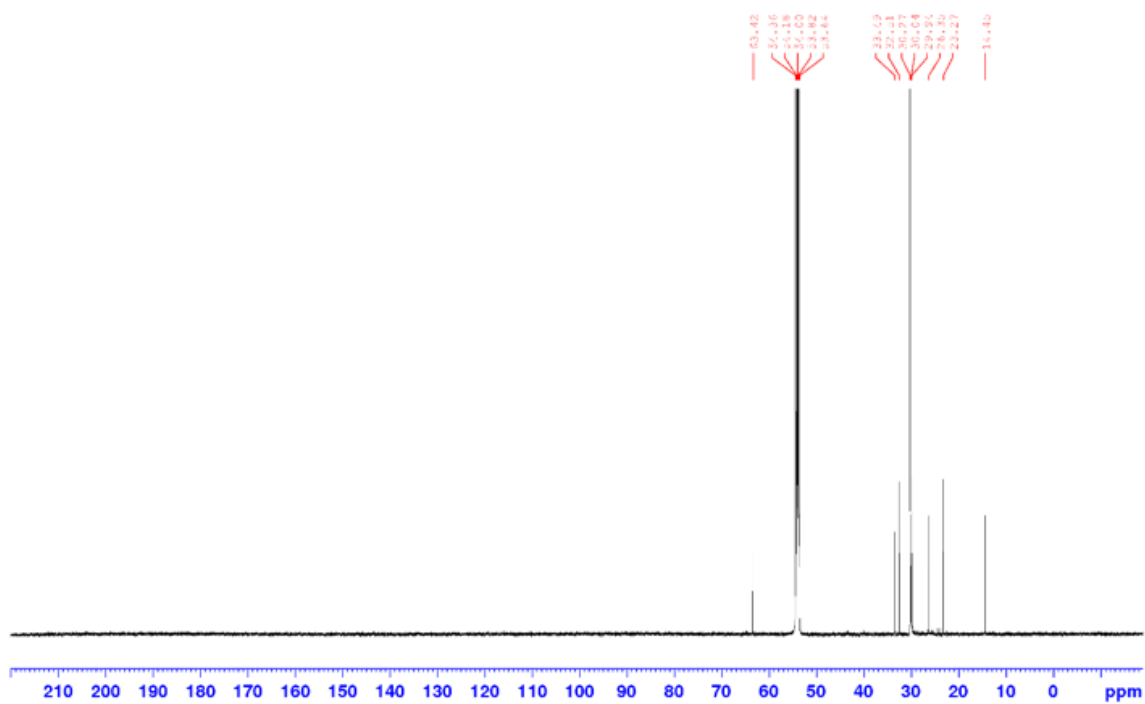
Compound 1
13C NMR (600MHz, CDCl3)

Figure S2. ^{13}C-NMR (150 MHz, CDCl$_3$) spectrum of compound 1 (Tetracosane)
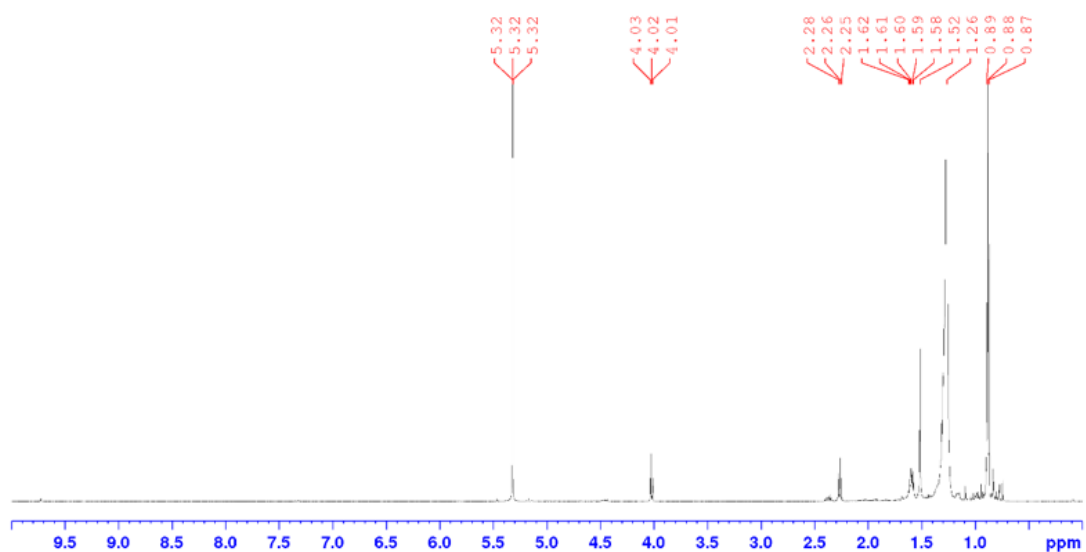
Figure S3. ¹H-NMR (600 MHz, CD$_2$Cl$_2$) spectrum of compound 2 (Hexadecyl palmitate)

Figure S4. ¹³C-NMR (150 MHz, CD$_2$Cl$_2$) spectrum of compound 2 (Hexadecyl palmitate)

Compound 3
1H NMR (600 MHz, CD2Cl2)

5.32
5.32
5.32

1.52
1.26
0.89
0.88
0.87
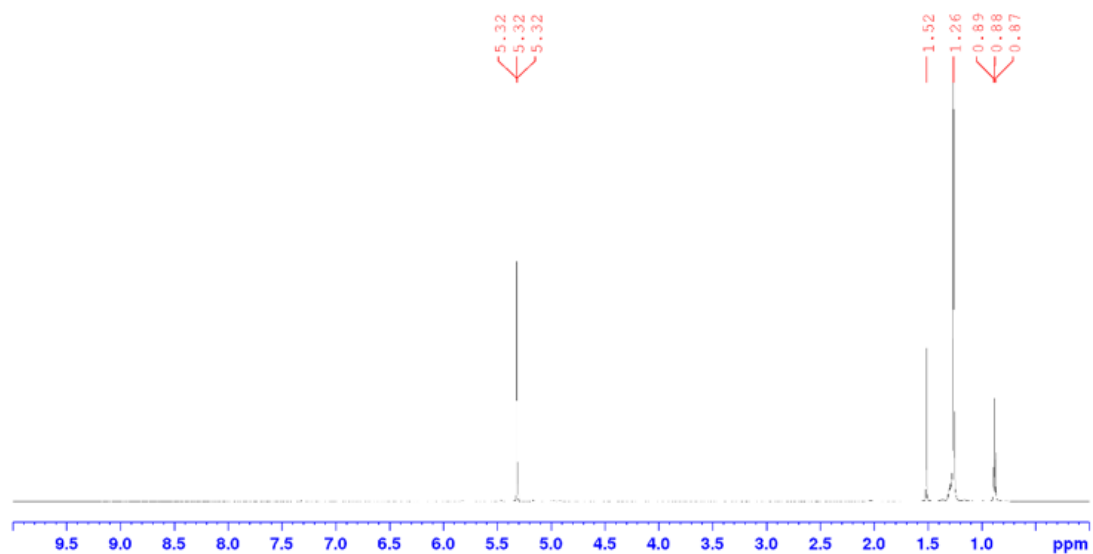
Figure S5: ^{1}H-NMR (600 MHz, CD$_2$Cl$_2$) spectrum of compound 3 (Octacosane)

Compound 3
13C NMR (150 MHz, CD2Cl2)
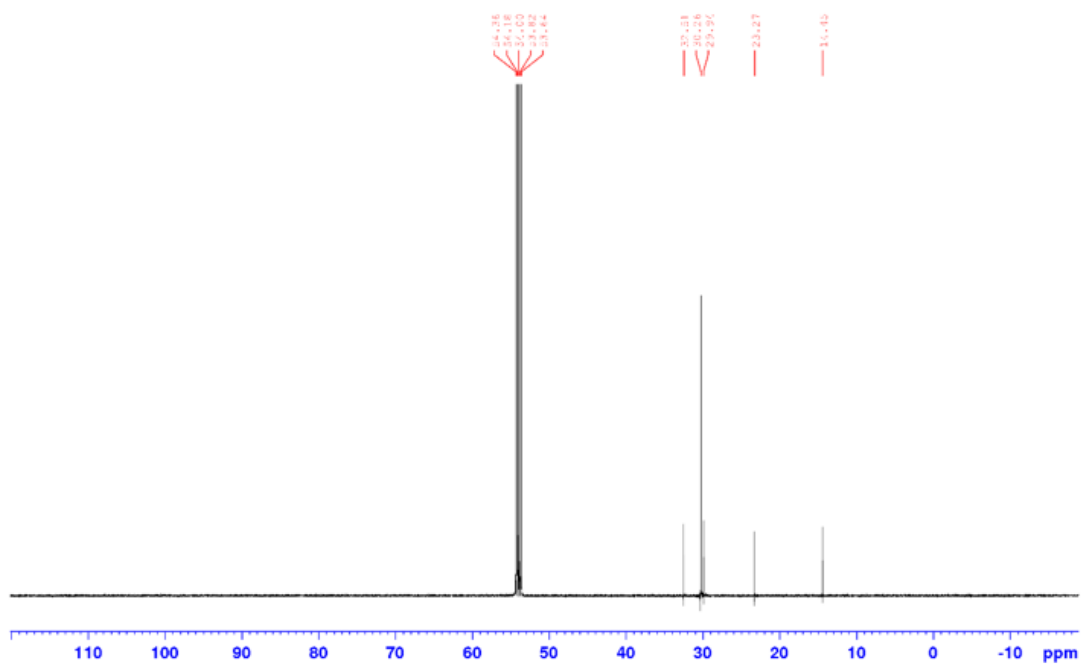
Figure S6: ^{13}C-NMR (150 MHz, CD$_2$Cl$_2$) spectrum of compound 3 (Octacosane)

Compound 4
1H NMR (600 MHz, CD2Cl2)
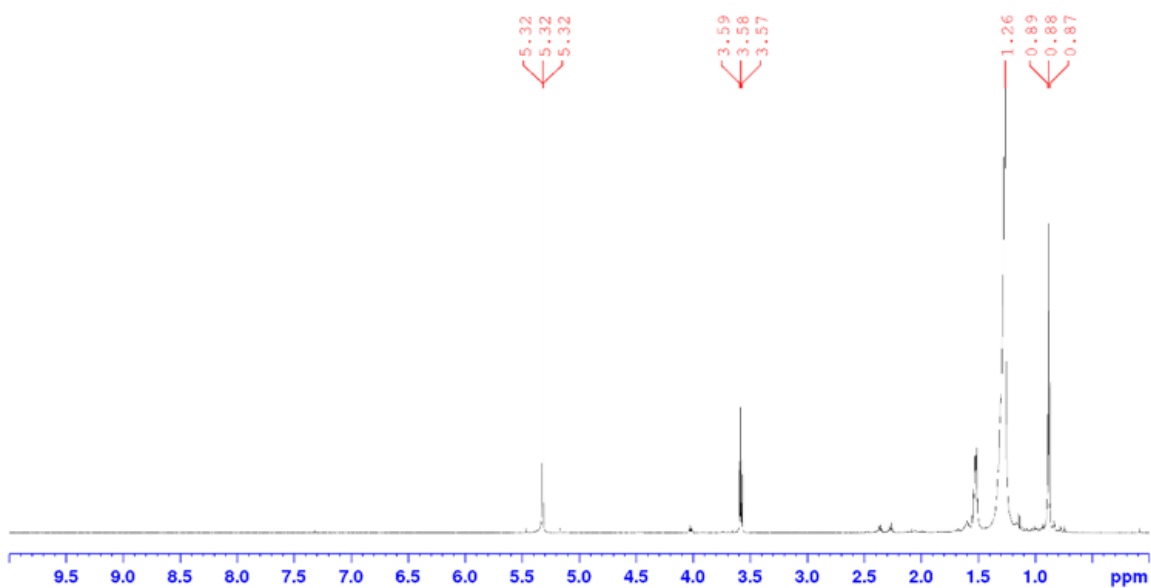
Figure S7. ^{1}H-NMR (600 MHz, CD$_2$Cl$_2$) spectrum of compound 4 (Tetracosanol)

Compound 4
13C NMR (150 MHz, CD2Cl2)

Figure S8. ^{13}C-NMR (150 MHz, CD$_2$Cl$_2$) spectrum of compound 4 (Tetracosanol)

Compound 5
1H NMR (600 MHz, CDCl3)
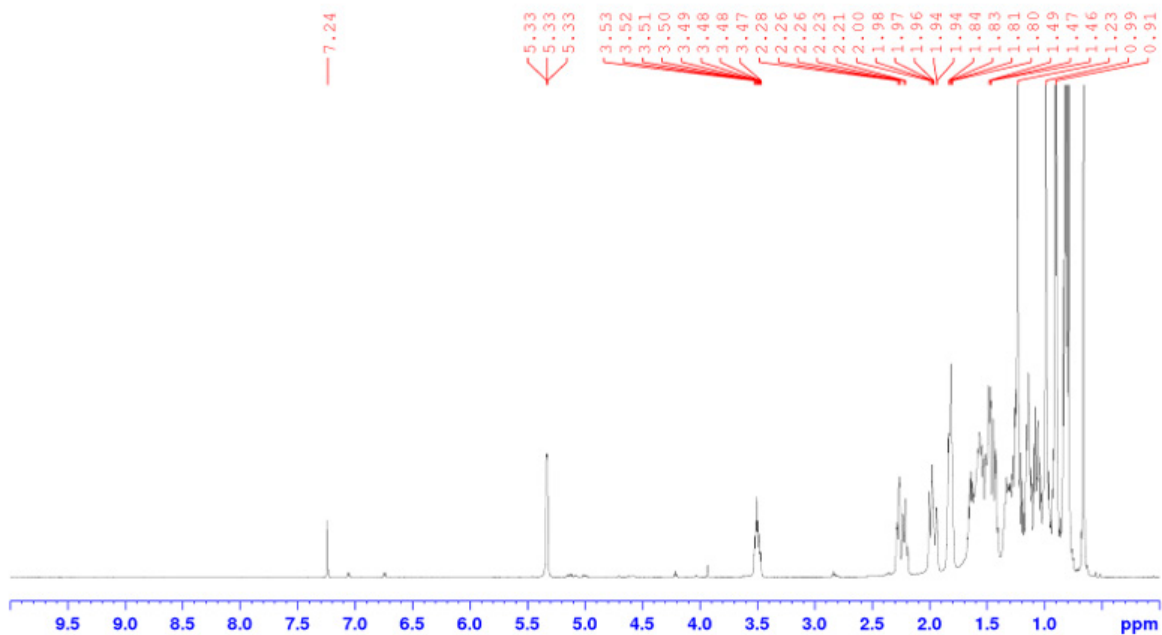
Figure S9. ^{1}H-NMR (600 MHz, CDCl$_3$) spectrum of compound 5 (β-sitosterol)
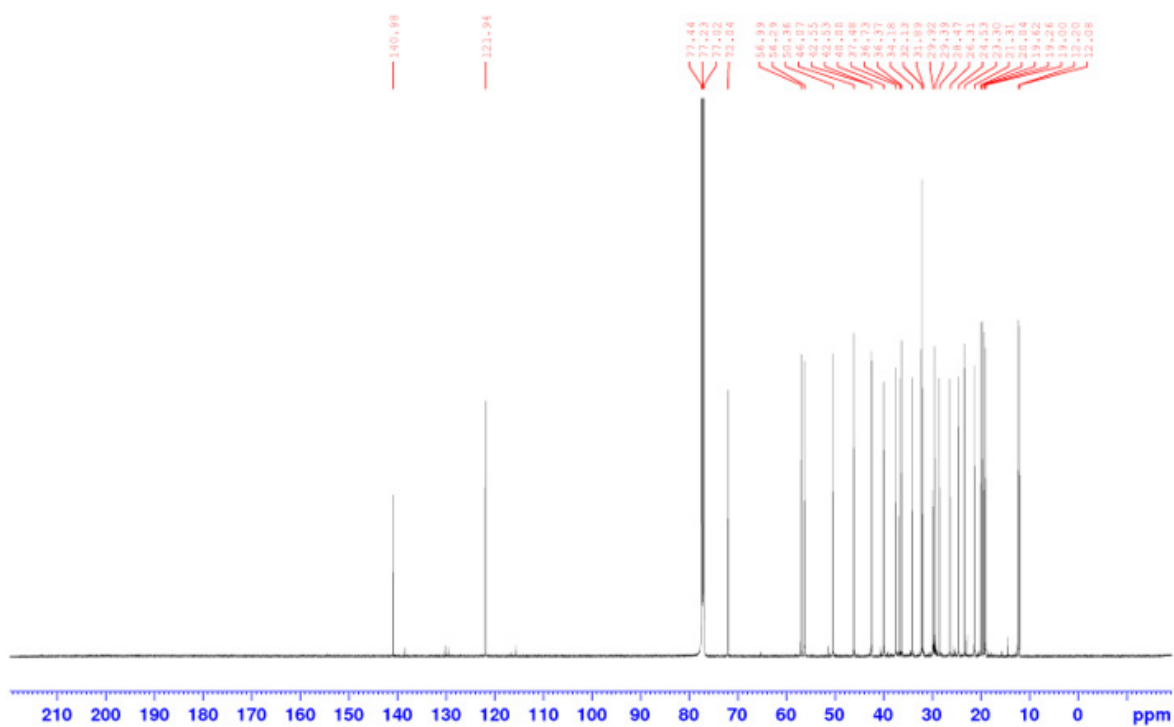
Compound 5
13C NMR (150 MHz, CDCl3)

Figure S10. ^{13}C-NMR (150 MHz, CDCl$_3$) spectrum of compound 5 (β-sitosterol)
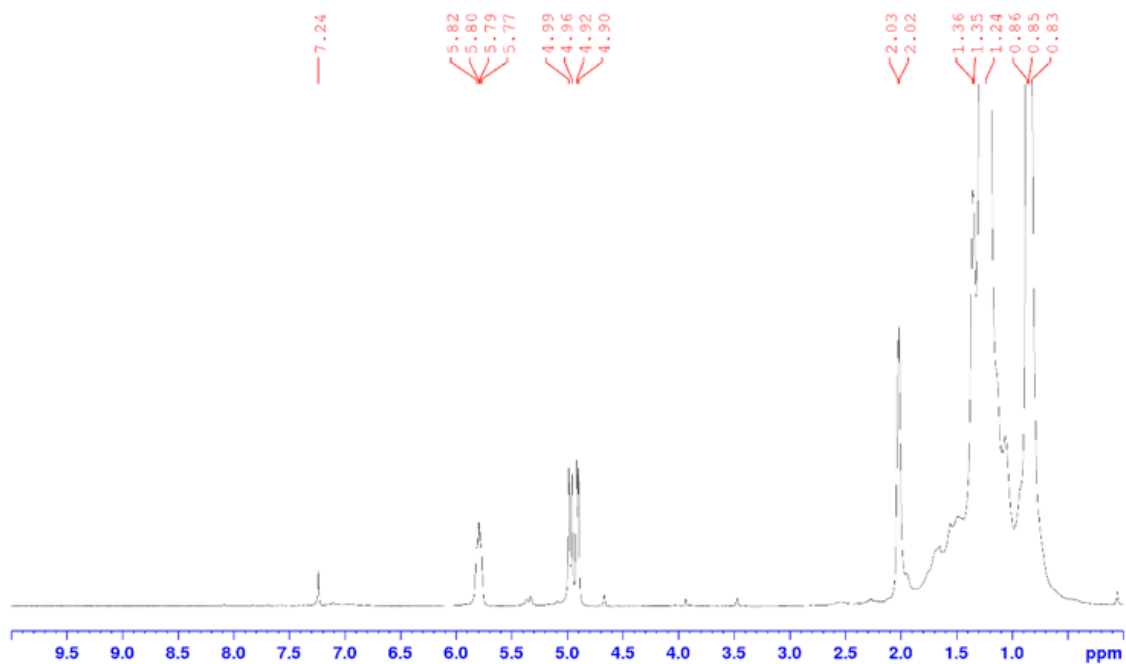
Compound 6
1H NMR (600 MHz, CDCl3)

Figure S11. ¹H-NMR (600 MHz, CDCl₃) spectrum of compound 6 (1-Hexacosene)

Compound 6
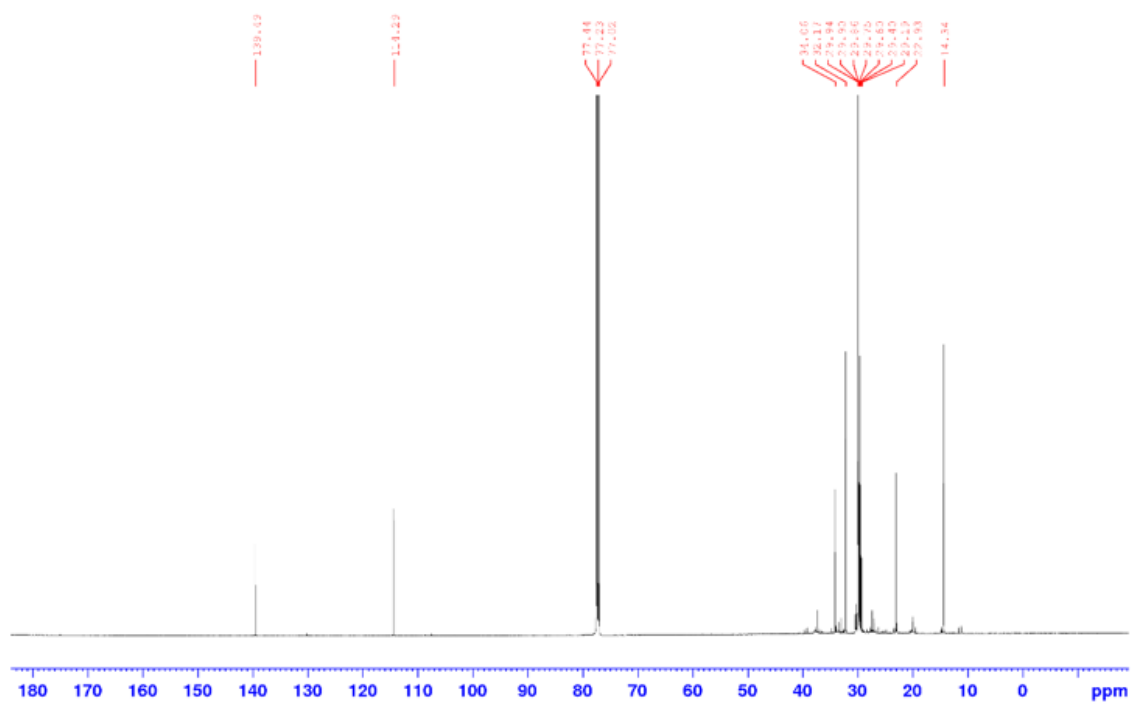13C NMR (150 MHz, CDCl3)

Figure S12. ¹³C-NMR (150 MHz, CDCl₃) spectrum of compound 6 (1-Hexacosene)
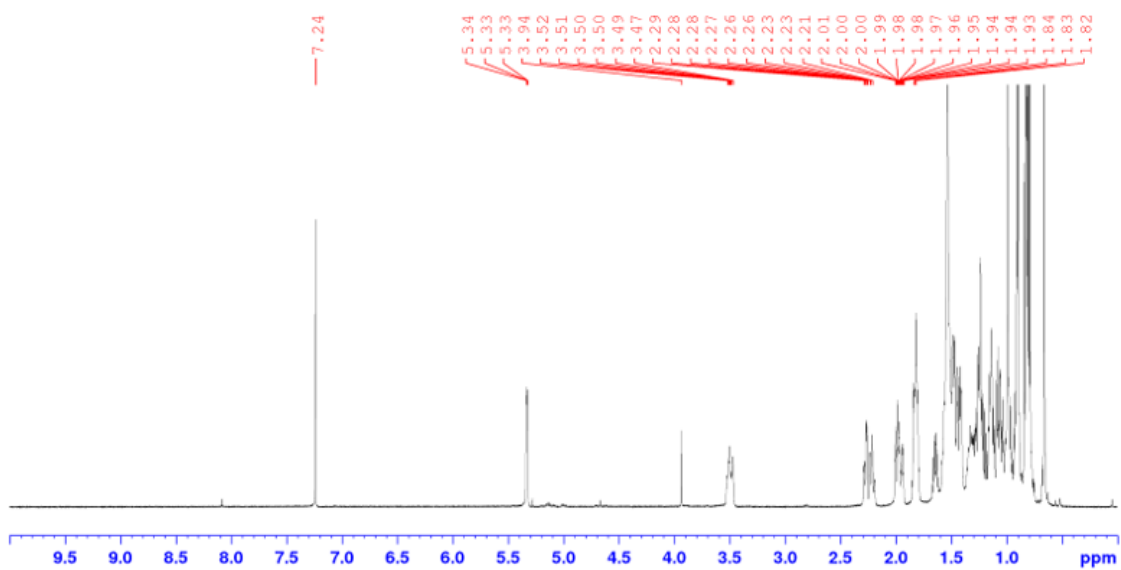
Compound 7
1H NMR (600 MHz, CDCl3)

Figure S13. ¹H-NMR (600 MHz, CDCl₃) spectrum of compound 7 (Campesterol)
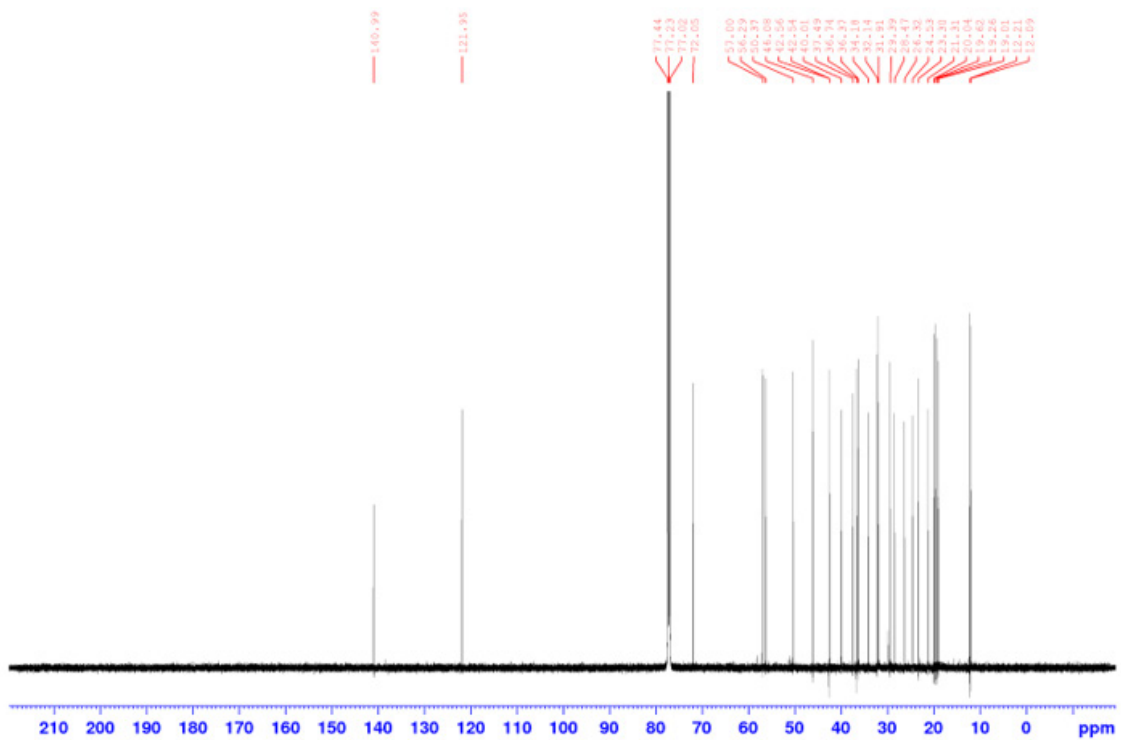
Compound 7
13C NMR (150 MHz, CDCl3)

Figure S14. ¹³C-NMR (150 MHz, CDCl₃) spectrum of compound 7 (Campesterol)
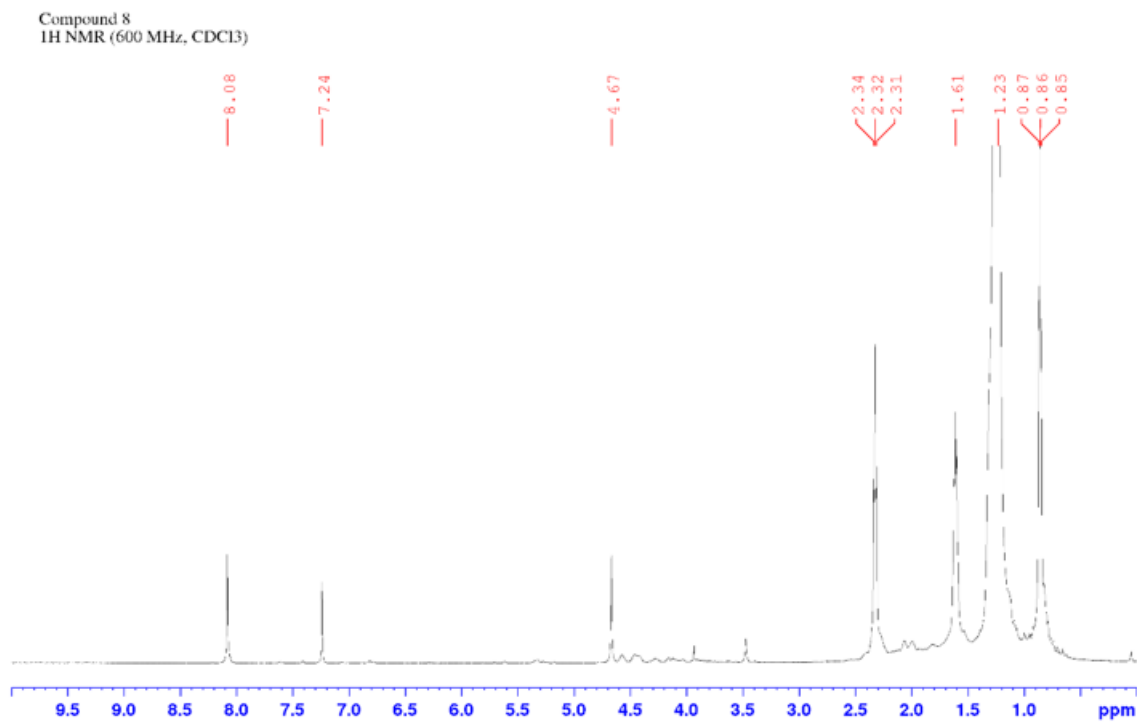
Compound 8
1H NMR (600 MHz, CDCl3)

8.08 7.24 4.67 2.34 2.32 2.31 1.61 1.23 0.87 0.86 0.85

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm

Figure S15. ^{1}H-NMR (600 MHz, CDCl$_3$) spectrum of compound 8 (Octadecanoic acid)

Compound 8
13C NMR (150 MHz, CDCl3)

178.97 77.44 77.23 77.02 34.25 32.3 29.92 29.81 29.66 29.58 29.48 29.25 25.22 24.92 22.91 14.33

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm
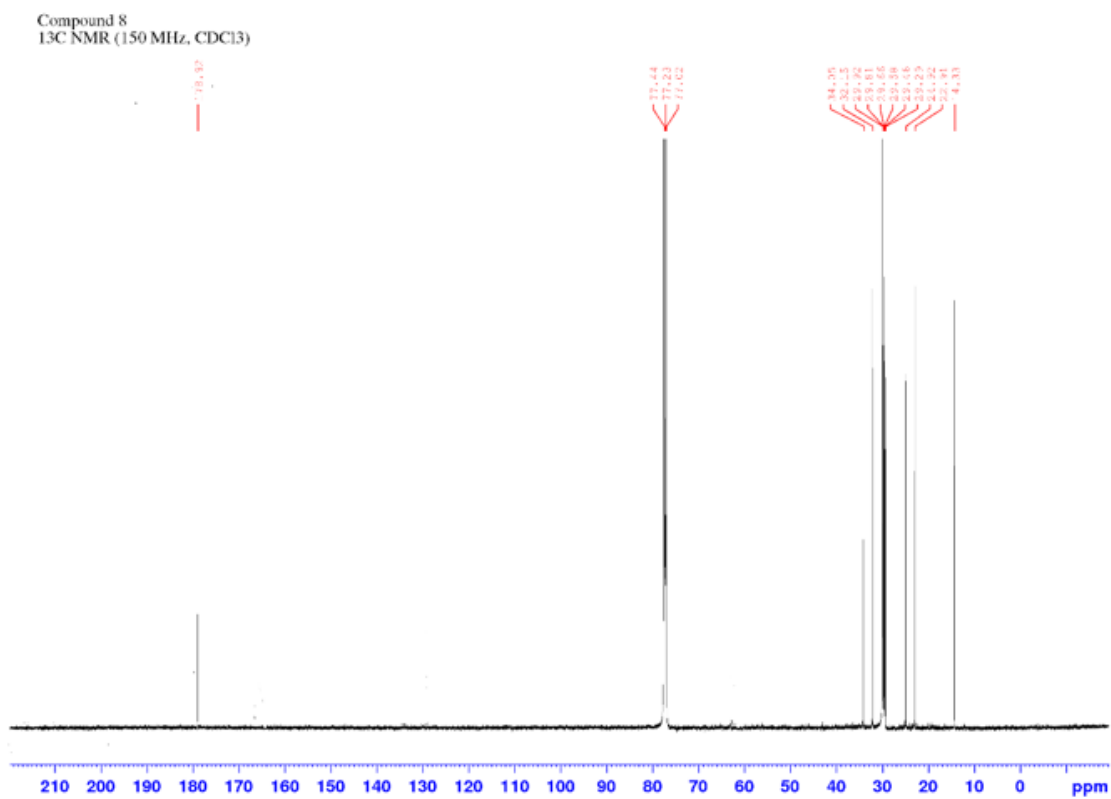
Figure S16. ^{13}C-NMR (150 MHz, CDCl$_3$) spectrum of compound 8 (Octadecanoic acid)
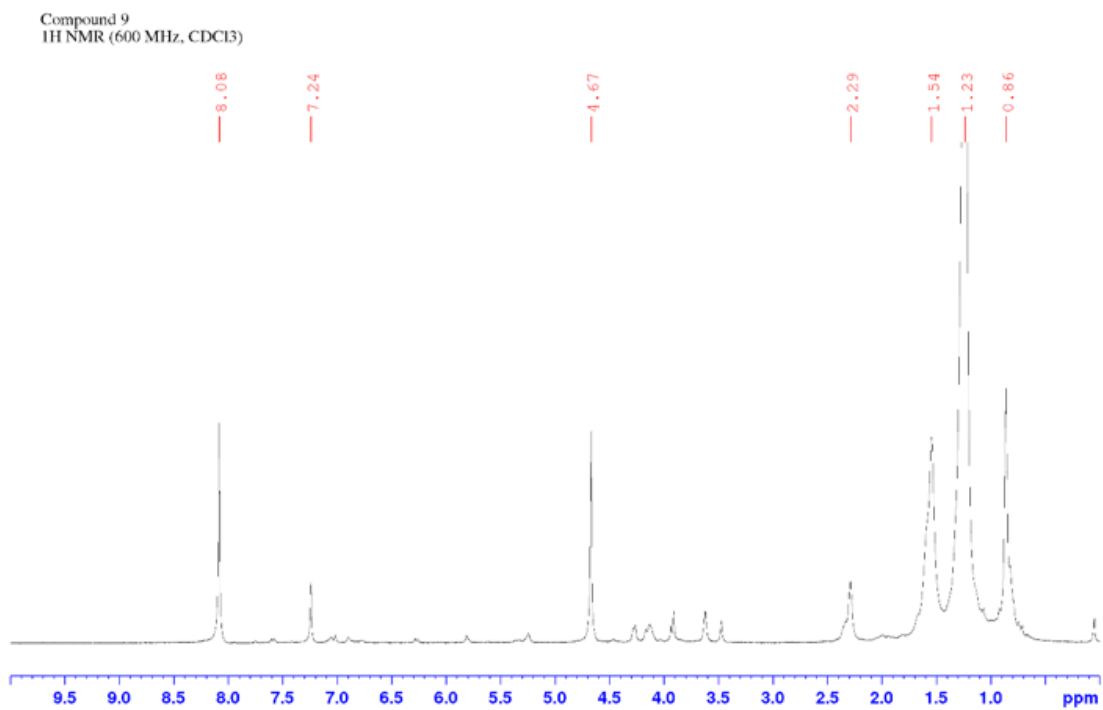
Compound 9
1H NMR (600 MHz, CDCl3)

Figure S17. ¹H-NMR (600 MHz, CDCl₃) spectrum of compound 9 (Tetracosanoic acid)

Compound 9
13C NMR (150 MHz, CDCl3)

Figure S18. ¹³C-NMR (150 MHz, CDCl₃) spectrum of compound 9 (Tetracosanoic acid)