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# Isolation and characterization of components from roots of *Premna latifolia* Roxb

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ABSTRACT

The aim of the current work was to isolate and characterize structurally fascinating and biologically intriguing compounds from the dried roots of *Premna latifolia*. Dried plant roots were subjected to soxhalation with ethyl alcohol and later to column chromatography. The individual compounds were isolated by preparative thin-layer chromatography followed by structural characterization using various spectral ways like LCMS, IR, 1D-NMR and 2D-NMR ($^1$H-$^1$H NMR and $^1$H-$^{13}$C NMR). Three totally different compounds were isolated and characterised as Compound 1: n-Tridecanyl n-Tetracosanoate, Compound 2: Stigmanstan -3$\beta$-olyln-octadec-9′, 12′-dienoate and Compound 3: n-Tetracosanol for the first time from *Premna latifolia* roots.

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## INTRODUCTION

Herbal medicines were used throughout human history and played a crucial role for the treatment of a wide variety of diseases and ailments. Ancient, Indian medicinal systems viz. Ayurveda, Siddha, Unani etc. and native health traditions provided a robust base for the use of medicinal plants in the management and treatment of various illness (Kayser *et al.*, 2000) . Herbal medication was additionally an efficient healing technique but was viewed less skyhigh (Jayaprasad *et al.*, 2012) . Herbal products were discarded from typical medicinal use, not essentially as a result of they were ineffective (Anbazhakan and Balu, 2007) but due to the reason that they weren't as economically profitable as the newer allopathic medications (Nagori *et al.*, 2011) . But now a day, the interest in "natural health" and therefore, the use of medicinal plants accrued steadily (Suresh *et al.*, 2011) . *Premna latifolia*, small bushy and thick trees, belonging to the Verbenaceae family (Yoganarasimhan, 2000) are spread across various Indian states like Kerala, Bihar, Karnataka, West Bengal, Assam etc. (Fletcher, 1938) . In vernacular languages, it is known as *Bakar* (Hindi), *Aginimantha* (Sanskrit), *Knappa* (Malayalam) and *Erumaimunnai* (Tamil) (Moldenke, 1983; Nadkarni, 1985; Krishnamurthi, 2005; Aravindakshan and Bai, 1996) . *Premna latifolia* is used as cariotonic, hepatoprotective, diuretic and antipyretic. Currently, two novel icetexanediterpenes and two new glycosides have been isolated from *Premna latifolia* (Ghosh *et al.*, 2014) ; (Thirumalai *et al.*, 2013) . As a part of continued efforts directed towards the study of the biologically active compounds from the various medicinal herbal plants, this work was designed and executed to isolate and characterize compounds from *Premna latifolia* roots (Wagner *et al.*, 1996) .

## MATERIALS AND METHODS

### Procurement and authentication of roots of *Premna latifolia*

Roots of *Premna latifolia* was purchased from a local market in Kasargod, Kerala, India and authenticated by NISCAIR New Delhi, India. [Authentication number–NISCAIR/RHMD/2011-12/1922/222/01]

### Extraction and isolation of compounds from dried roots

The coarsely pulverised dried roots of *Premna latifolia* (5kg) were subjected to soxhlet extraction with absolute ethyl alcohol (50 liters) (Silverstein *et al.*, 2005) . The concentrated extract was later subjected to gradient elution by using column chromatography where, methanol, chloroform, ether were used in variable proportions (Brain *et al.*, 1985) . The fractions of the plant were collected on an individual basis and combined based on TLC pattern (Harbone, 1998; Ali, 2008) . The individual compounds were further separated and sublimated using preparative TLC and Toluene: ethyl acetate (9.3:0.7) as mobile phase (Gupta *et al.*, 2008) . The isolated compounds were characterized using FTIR, LC-MS, 1H NMR, 13C NMR and 2D-NMR techniques (Parameswari *et al.*, 2015; Kamalambigeswari *et al.*, 2018) .

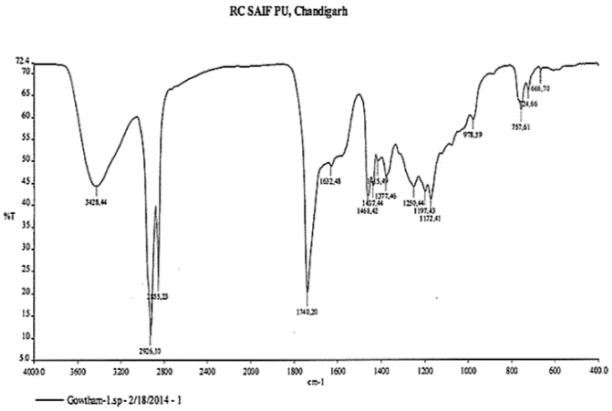


**Figure 1: FTIR of Compound 1**

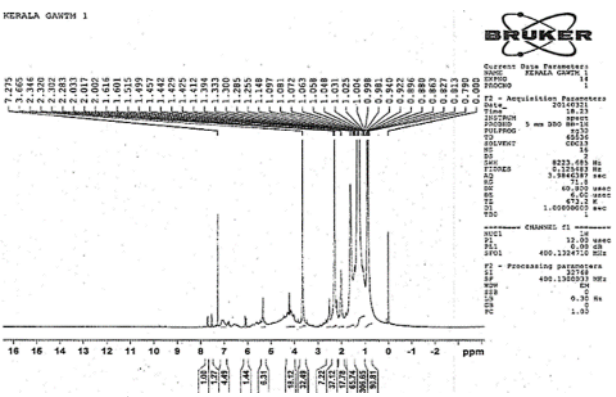


**Figure 2: $^{1}$H NMR of Compound 1**

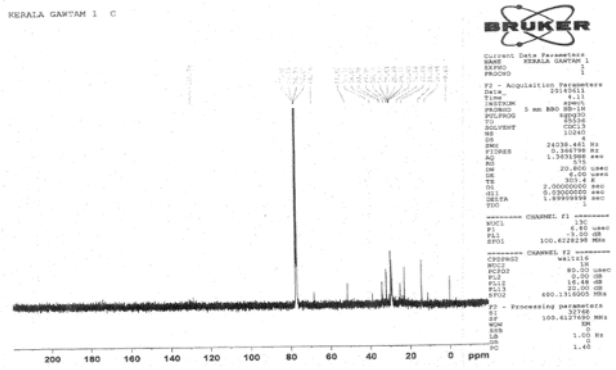


**Figure 3: $^{13}$C NMR of Compound 1**

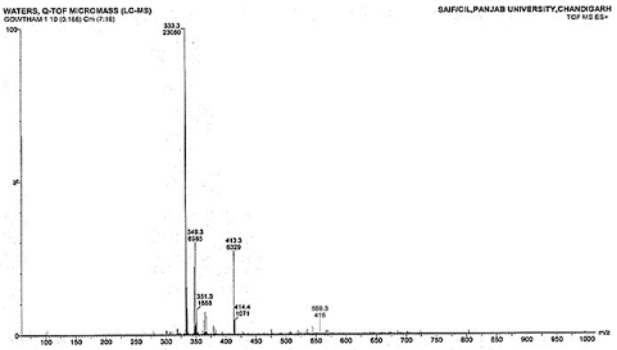


**Figure 4: Mass spectra of compound 1**

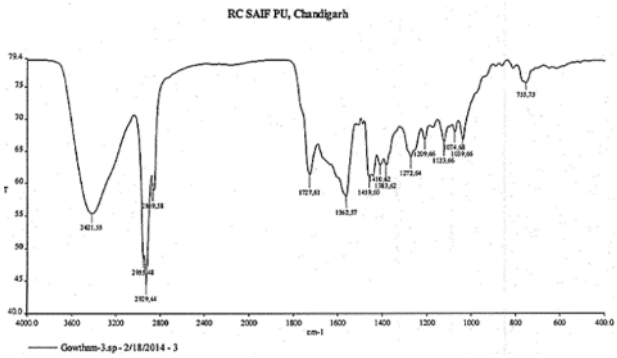


**Figure 5: FTIR of Compound 2**

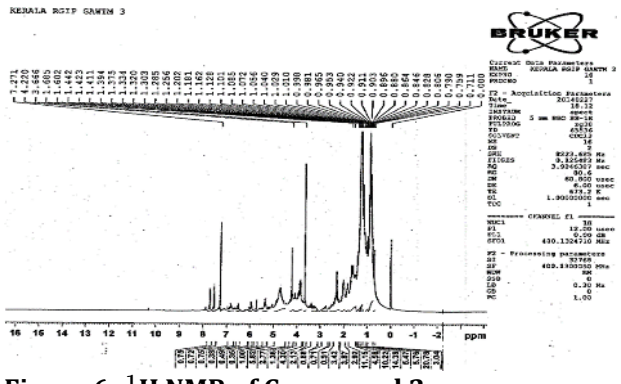


**Figure 6: $^{1}$H NMR of Compound 2**

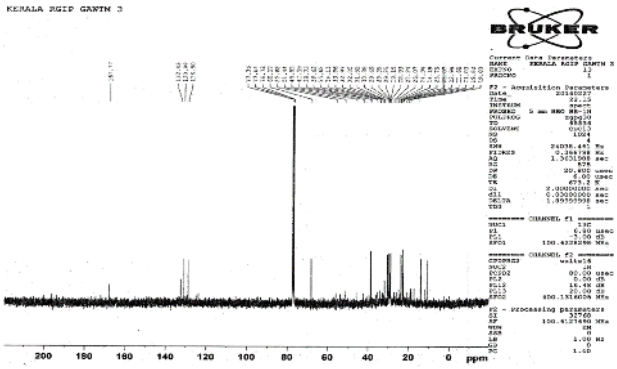

**Figure 7: $^{13}$C NMR of Compound 2**

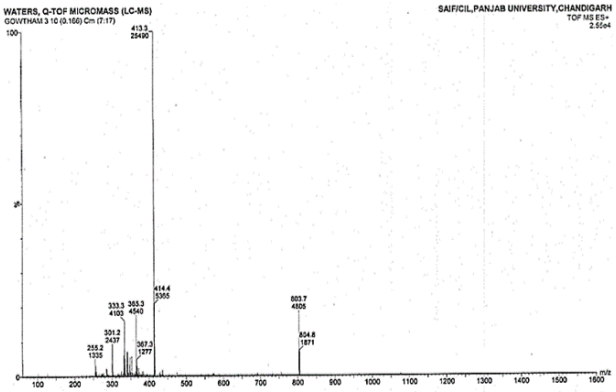

**Figure 8: Mass spectra of Compound 2**

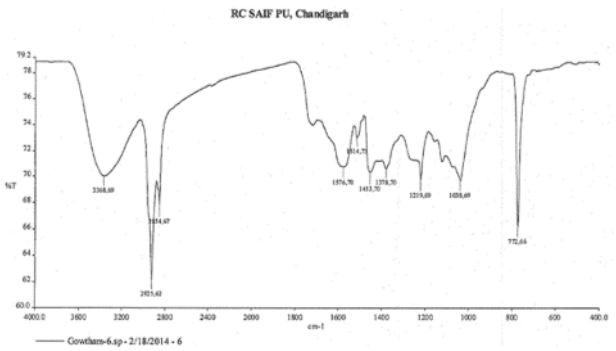

**Figure 9: FTIR of Compound 3**

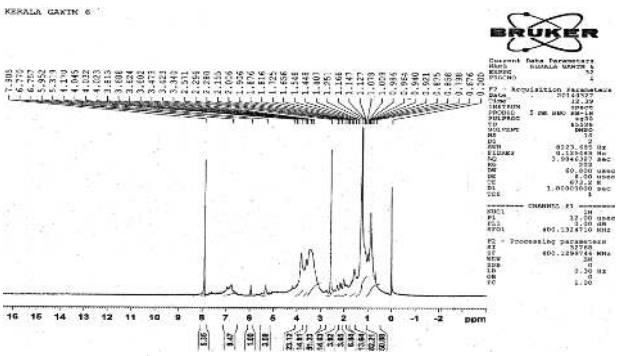

**Figure 10: $^{1}$H NMR of Compound 3**

**Compound 1: n-Tridecanyl n-Tetracosanoate**

When the column was eluted with chloroform 100%,it resulted in a reddish brown solid mass (Gowtham *et al.*, 2018) ; (Asharani *et al.*, 2013) of compound 1 of yield 1.352 g (0.233% yield) with $R_f$ value 0.62 (Toluene: Ethylacetate (9.3:0.7)); m.p.57-59°C; IR $\lambda$ max (KBr): 2926, 2855, 1740, 1632, 1461, 1377, 1250, 1197,1172, 757cm$^{-1}$ (Figure 1 );$^{1}$H NMR ($CDCl_3$): $\delta$ 2.01 (2H, m, $CH_2$), 2.28 (1H, t, J=7.2 Hz, $H_2$-2), 2.21 (2H, m, $CH_2$), 1.68 (2H, m, $CH_2$), 1.60 (2H, m, $CH_2$), 1.51 (2H, m, $CH_2$), 0.89 (3H, t, J=6.4 Hz, Me-24), 1.30 (8H, brs, 4 x $CH_2$), 1.28 (2OH, brs, 10 x $CH_2$), 1.25 (18H, brs, 9 x $CH_2$), 1.14 (8H, brs, 4 x $CH_2$), 4.27 (1H, t, J=6.2 Hz, $H_2$-1′), 0.86 (3H, t, J=6.8 Hz, Me-13′) (Figure 2 ); $^{13}$C NMR ($CDCl_3$): $\delta$ 172.69 (OCO), 64.56 ($CH_2$-1′), 51.40 ($CH_2$-2), 38.78 ($CH_2$), 34.05 ($CH_2$), 28.83 ($CH_2$), 33.70 ($CH_2$), 31.90 ($CH_2$), 30.90 ($CH_2$), 29.67 (21 x $CH_2$), 29.63 ($CH_2$), 29.33 ($CH_2$), 29.12 ($CH_2$), 10.93 (Me-13′), 26.04 ($CH_2$), 22.66 ($CH_2$), 14.06 (Me-24) (Figure 3 ); LC-MS m/z (rel. int.): 414 $[M]^+$ ($C_{37}H_{74}O_2$) (1.8), 334 (4.8), 350 (4.3) (Figure 4 ).

**Compound 2: Stigmanstan -3$\beta$-olyln-octadec-9′, 12′-dienoate**

Further when column was eluted with chloroform 100%, it gave a brownish black solid mass (Gowtham *et al.*, 2018) of compound 2 of yield 1.260 mg (0.217% yield) with $R_f$ value 0.31 (Toluene: Ethylacetate (9.3:0.7)); m.p.102-104°C; IR $\lambda$ max (KBr): 2929, 2862, 1721, 1645, 1459, 1383, 1272, 1209, 1123, 1039, 755 cm$^{-1}$ (Figure 5 ); $^{1}$H NMR ($CDCl_3$): $\delta$ 4.71 (1H, brm, $w_{1/2}$ 16.8 Hz, H-3$\alpha$), 5.36 (1H, m, H-10′), 2.03 (2H, m, $H_2$-8′), 0.71 (3H, brs, Me-18), 5.32 (1H, m, H-12′), 5.17 (1H, m, H-9′), 2.31 (2H, t, J= 7.2 Hz, $H_2$-2′), 5.04 (1H, m, H-13′),1.56 (2H, m, $CH_2$-6), 2.35 (1H, m, $H_2$-11′), 1.94 (2H, m, $H_2$-14′), 1.89 – 1.60 (25H, m, 9 x $CH_2$, 7 x CH), 0.80 (3H, t, J= 6.3 Hz, Me-29), 1.42 (1H, m, H-5), 0.89 (3H, d, J= 6.4 Hz, Me-27), 1.33 (2H, m, $CH_2$), 0.86 (3H, J= 7.2 Hz, Me-18′), 1.30 (6H, brs, 3 x $CH_2$), 1.28 (6H, brs, 3 x $CH_2$), 1.25 (8H, brs, 4 x $CH_2$), 1.18 (3H, brs, Me-19), 0.95 (3H, d, J= 6.2 Hz, Me-21), 0.92 (3H, d, J= 7.2 Hz, Me-26) (Figure 6 ); $^{13}$C NMR ($CDCl_3$): $\delta$ 33.11 (C-1), 55.88 (C-14), 68.17 (C-3), 38.73 (C-4), 22.98 (C-6), 34.11 (C-8), 24.94 (C-16′), 30.36 (C-2), 32.05 (C-7), 51.44 (C-9), 35.62 (C-10), 21.03 (C-11), 39.06 (C-12), 42.39 (C-13), 24.19 (C-15), 130.88 (C-12′), 27.24 (C-16), 55.83 (C-17), 10.96 (C-18), 18.70 (C-19), 38.62 (C-20), 17.39 (C-21), 32.97 (C-22), 23.75 (C-23), 45.83 (C-24), 31.92 (C-25), 39.81 (C-5), 19.03 (C-26), 19.82 (C-27), 23.07 (C-28), 11.97 (C-29), 167.77 (C-1′), 25.56 (C-15′), 29.69 (C-2′), 28.93 (C-6′), 22.68 (C-17′), 29.35 (C-4′), 132.45 (C-10′), 29.15

(C-7′), 29.55 (C-8′), 33.96 (C-11′), 29.15 (C-5′), 121.26 (C-13′), 29.61 (C-3′), 29.25 (C-14′), 14.11 (C-18′) (Figure 7 ); LC-MS m/z (rel. int.): 805 $[M]^+$ ($C_{47}H_{82}O_2$) (1.2), 415 (25.3), 366 (16.6) (Figure 8 ).

**Compound 3: n-Tetracosanol**

On continuing the elution with chloroform 100%, it furnished a dark black solid mass of compound 3 of yield 1.820 mg (0.313% yield) with $R_f$ value 0.82 (Toluene: Ethylacetate (9.3:0.7)); m.p.75-77°C; IR $\lambda$ max (KBr): 3368, 2925, 2854, 1453, 1378, 1219, 1038, 773 $cm^{-1}$ (Figure 9 ); $^1H$ NMR ($CDCl_3$): $\delta$ 1.25 (38H, brs, 19 x $CH_2$), 3.42 (2H, t, J=9.5 Hz, $H_2$-1), 1.54 (4H, m, 2 x$CH_2$), 0.85 (3H, t, J=6.5Hz, Me-24), 1.87 (2H, m, $CH_2$) (Figure 10 ); $^{13}C$ NMR ($CDCl_3$): $\delta$ 64.15 ($CH_2$-1), 35.68 ($CH_2$), 33.42 ($CH_2$), 22.68 ($CH_2$), 31.26 ($CH_2$), 29.06 (12 x $CH_2$), 27.36 (CH2), 28.93 ($CH_2$), 28.81 ($CH_2$), 14.16 (Me-24)28.63 ($CH_2$), 26.51 ($CH_2$), 25.13 ($CH_2$) (Figure 11 ); LC-MS m/z (rel. int.): 354 $[M]^+$ ($C_{24}H_{50}O$) (11.2) (Figure 12 ).

## RESULTS AND DISCUSSION

This work resulted in the isolation of three novel compounds from dried roots of *Premna latifolia* Roxb.

**Compound 1: n-Tridecanyl n-Tetracosanoate**

Compound 1, a fatty ester, showed bands for ester group (1740 $cm^{-1}$) and long aliphatic chain in IR absorption spectra. Its mass spectra exhibited a molecular ion peak at mass/charge 550 which complements to a molecular formula $C_{37}H_{74}O_2$, a fatty ester. The ion peaks emerging at m/z 351($C_1$-O fission, $[CH_3\ (CH_2)_{22}CO]^+$) and 367 ($C_1$′-O fission, $[CH_3(CH_2)_{22}COO]^+$) designated that lignoceric acid and n-tridecanol were esterified together. The $^1$HNMR spectra of compound 1 displayed proton triplet at $\delta$ 4.27 (J=6.0 Hz) assigned to oxygenated methylene $H_2$-1′ proton. A two proton triplet in the upfield region at $\delta$ 2.28 (J= 7.2 Hz) was associated to methylene $H_2$-2 proton. Other protons of methylene resonated between $\delta$ 2.20- 1.14. The proton triplets at $\delta$ 0.89 (J= 6.4 Hz) and 0.86 (J= 6.8 Hz) were accounted to terminal C-24 and C-13′ primary methyl protons. $^{13}$CNMR spectra of compound 1 showed ester carbon signals at $\delta$ 172.61 (C-1), oxygenated methylene carbon at $\delta$ 64.56 (C-1′), other methylene carbons from $\delta$ 51.40 to 22.66 and methyl carbons at $\delta$ 14.0 (C-24) and 10.93 (C-13′). The $^1$H-$^1$H COSY spectra exhibited correlations of $H_2$- 22 and $H_2$- 23 with Me- 24; $H_2$-1′ with $H_2$-2′ and $H_2$-3′; and $H_2$- 12′ with Me-13′. The HMBC spectra of compound 1 indicated interaction of $H_2$-2′ and $H_2$-3′ with C-1′; $H_2$-23 with C-24; $H_2$-2 and $H_2$-23 with C-1 and $H_2$-12′ with C-1′. Based on the above data, the name of Compound 1 is suggested as-n-Tridecanyl n-Tetracosanoate (Figure 13 ).

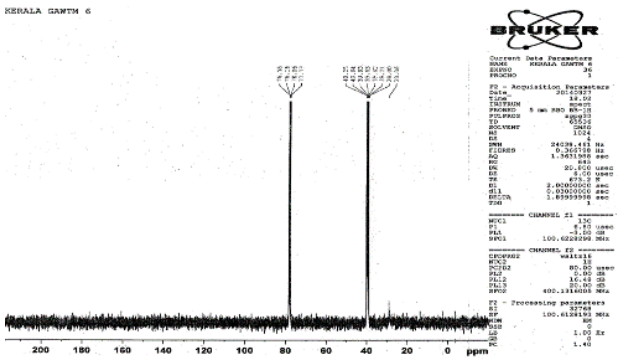


**Figure 11: $^{13}$C NMR of Compound 3**

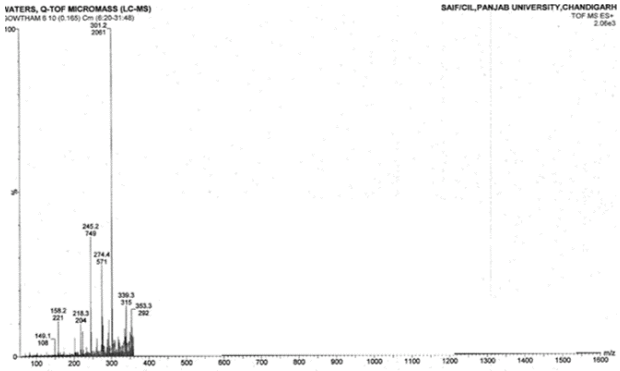


**Figure 12: Mass spectra of Compound 3**

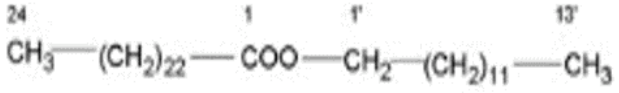


**Figure 13: Structure of n-Tridecanyl n-Tetracosanoate.**

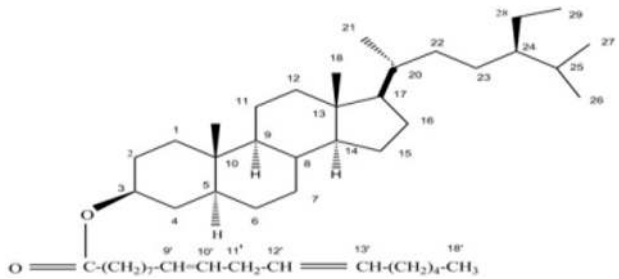


**Figure 14: Structure of Stigmanstan -3$\beta$-olyln-octadec-9′, 12′-dienoate.**

**Compound 2: Stigmanstan -3$\beta$-olyln-octadec-9′, 12′-dienoate**

IR spectra displayed characteristic bands attributed for unsaturation (1645 $cm^{-1}$), ester group (1721 $cm^{-1}$) and aliphatic chain, which is long (755 $cm^{-1}$). From mass spectra, the molecular ion peak was determined at m/z 678, and $^{13}$C NMR spectra

 

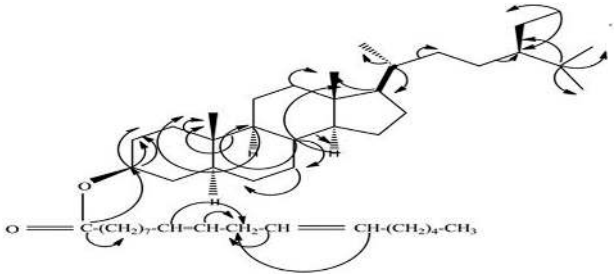

**Figure 15: HMBC correlations of Stigmanstan -3$\beta$-olyln-octadec-9′, 12′-dienoate.**

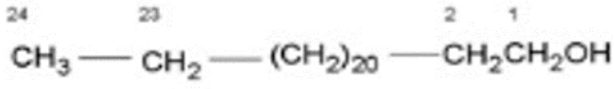


**Figure 16: Structure of n-Tetracosanol**

were correlated with the molecular formula of a sterol ester $C_{47}H_{82}O_2$. The peaks arising at m/z 415 [CO-O fission, $C_{29}H_{51}O]^+$ and 279 $[M]^+$- 415, $CH_3(CH_2)_7CH{=}CHCH_2{-}CH{=}CH(CH_2)_4COOH]^+$ accredited that sterol was esterified with the linoleic acid. The $^1$H NMR spectra of compound 2 displayed four one-proton multiplets from $\delta$ 5.36 to 5.04 adduced to vinylic H-9′, H-10′, H-12′ and H-13′ protons. A multiplet one-proton broad at $\delta$ 4.71 with a half-width of 16.8 Hz was associated to oxygenated methine H-3 proton ($\alpha$-oriented). A 2 proton multiplet and triplet at $\delta$ 2.35 and $\delta$ 2.31 (J=7.2 Hz) respectively, were due to methylene $H_2$-11′ between two vinylic carbons and to methylene $H_2$-2′ protons which were adjacent to the ester function. An 3- proton doublets at $\delta$ 0.95 (J=6.2 Hz), 3-proton triplets at $\delta$ 0.86 (J= 7.2 Hz) and 0.80 (J= 6.3 Hz) and 3 proton broad singlets at $\delta$ 1.18 and 0.71, 0.92 (J=7.2 Hz), 0.89 (J=6.4 Hz) were assigned with tertiary C-19 and C-18 secondary C-21, C-26 and C-27 and primary C-18′ and C-29 methyl protons, respectively, all attached to the saturated carbons. The remaining methylene and methine protons resonated from $\delta$ 2.03- 1.25. The $^{13}$C NMR spectra of compound 2displayed presence for vinylic carbons at $\delta$ 126.90 (C-9′), 132.45 (C-10′), 130.88 (C-12′) and 121.26 (C-13′), oxygenated methylene carbon at $\delta$ 68.17 (C-3), ester carbon at $\delta$ 167.77 (C-1′) and methyl carbons between $\delta$ 18.70 –10.96. The $^1$H-$^1$H COSY spectra of compound 2 showed correlations of H-3 with $H_2$-1, $H_2$-2, $H_2$-4 and H-5; H-8 with $H_2$-6, $H_2$-7, H-9 and H_14; H-17 with $H_2$-16, $H_2$-22, H-20 and Me-21; H-24 with $H_2$-23, $H_2$-28, H-25, Me-26, Me-27 and Me-29; and $H_2$-11 with H-9′, H-10′, H-12′ and H-13′. The HMBC spectra of compound 2 exhibited associations of H-3 and $H_2$-2′ with C-1′; $H_2$-1, $H_2$-2, $H_2$-4 and H-5 with C-3; $H_2$-1, H-5, H-9 with C-19; H-8 with $H_2$-6, $H_2$-7, H-9 and H_14; $H_2$-12, H-14, and H-17 with C-18; H-17, $H_2$-22 and Me-21 with C-20; and $H_2$-23, $H_2$-28, H-25, Me-26, Me-27 and Me-29 with C-24. The $^{13}$C NMR and $^1$H spectral data of the steroidal nucleus obtained were compared with all the reported spectral steroidal values. Based on these information, the structure of compound 2 was elucidated and confirmed as stigmanstan -3$\beta$-olyl *n*-octadec-9′, 12′-dienoate (Figure 14 ) , (Figure 15 ). This is a new sterol ester.

**Compound 3: n-Tetracosanol**

The IR spectra of compound 3 showed at 3368 $cm^{-1}$, which suggest the presence of OH group along with a long aliphatic chain at 773 $cm^{-1}$. The mol. ion peak at m/z 354 $[M]^+$ in mass spectra exhibited a molecular formula $C_{24}H_{50}O$. The $^1$H NMR spectra showed the presence of a two proton triplet at $\delta$ 3.42(J= 9.5 Hz) and a 3-proton triplet at 0.85 (J= 6.5 Hz) adduced to hydroxy methylene $H_2$-1 and terminal C-24 primary methyl protons respectively. The methylene protons were found to resonate as multiplets at $\delta$ 1.87 (2H) and $\delta$ 1.54 (4H) and as abroad singlet at $\delta$ 1.25 (38H). The $^{13}$C NMR spectra of compound **3** displayed signals for hydroxy methylene carbon at $\delta$ 64.15 (C-1), methylene carbons between $\delta$ 35.68- $\delta$ 22.68 and methyl carbon at $\delta$ 14.16 (C-24). The $^1$H - $^1$H COSY spectra showed correlations of $H_2$- 1 with $H_2$- 2 and $H_2$-3 and Me-24 with $H_2$ – 23 and $H_2$ -22 respectively. The HMBC spectra of compound 3exhibited interactions of $H_2$- 2 and $H_2$-3 with C-24. Based on the above datas and discussion, the structure of compound **3** has been confirmed as n-Tetracosanol (Figure 16 ).

## CONCLUSIONS

Plants are blessed with the ability to produce a large number of diverse bioactive and interesting compounds with important pharmacological activities. The present work led to isolation and characterization three compounds, namely Compound 1: n-Tridecanyl n-Tetracosanoate, Compound 2: Stigmanstan -3$\beta$-olyln-octadec-9′, 12′-dienoate and Compound 3: n-Tetracosanol which possess the significant pharmacological activity and can be drugs of future.

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