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Phytochemical Studies on Linum Usitatissimum Seeds and the Nanoformulation of the Bioactive Butanol Extract

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Abstract:

The phytochemical investigation of *Linum usitatissimum*, family Linaceae, resulted in the separation and identification of a lignane, 8–5' neolignan **1** (dehydrodiconiferyl alcohol-4-β-D-glucoside), in addition to identification of many compounds by the GC/MS technique. The antimicrobial activities of hexane, methylene chloride and butanol fractions were comparable to ampicillin. The activities against *E. coli* were 29.2%, 37.5%, and 66.7%, respectively; against *S. aureus* were 45.4%, 36.4% and 63.6%, respectively, and against *C. albicans* were 26.9%, 46.1% and 73.1%, respectively. Antioxidant activity was assessed by ABTS method. It decreased in the following order: ascorbic acid > butanol fraction > methylene chloride fraction > hexane fraction. The cytotoxicity against HePG2 was found to be “moderate” for butanol and methylene chloride fractions, and “weak” for the hexane fraction. The cytotoxicity against MCF-7 was found to be “strong” for butanol fraction, and “weak” for both hexane and methylene chloride fractions. The lignane-rich subfraction, Lu 3d was incorporated into pluronic nano-micelles using nanoprecipitation technique through a modified procedure. The physicochemical characteristics of the developed Lu 3d-loaded nano-micelles such as particle size, potential and morphology were determined using DLS and HR-TEM. The average diameters of the prepared plain and Lu 3d-loaded pluronic nano-micelles were found to be 207 ± 12 and 225 ± 18 nm, respectively. The encapsulation of Lu 3d into nano-sized particles has enhanced their aqueous dissolution and consequently improved their bioavailability. Nanoformulation of Lu 3d led also to a stable colloidal dispersion with a strong green color, indicating its homogenous distribution in the aqueous medium at a significantly higher concentration than that obtained using other solvents.

Keywords: *Linum usitatissimum;* Linaceae; Flax; 8-5’ neolignan; pluronic nano-micelles; controlled release.

1. INTRODUCTION

Linum usitatissimum L., flaxseed or linseed, is one of the most ancient of cultivated crops **[1]** of the family Linaceae. It is a food and fiber crop that is grown in cooler regions of the world.Linaceae family includes approximately 250 species. There are 14 genera, classified into two subfamilies: Linoideae and Hugonioideae (often recognized as a distinct family, the Hugoniaceae). Flax was first domesticated in the Fertile Crescent region **[2]**.

The agronomic value of the plant lies in the seeds; it has the ability to reduce the risk of definite diseases **[3]**. It contains nearly 38–40% fat, 23–25% proteins and 15–20% carbohydrates of which one quarter to one-third consisted of mucilage **[4]**.

Flaxseed is the richest dietary source of lignans. The major flaxseed lignan, is secoisolariciresinol, in the form of secoisolariciresinol diglucoside. The phytochemical investigation of *L. usitatissimum* revealed the presence of steroids **[5]**, flavonoids **[6]**, lignans **[7]**, phenolics **[8]** and fatty acids **[9].**

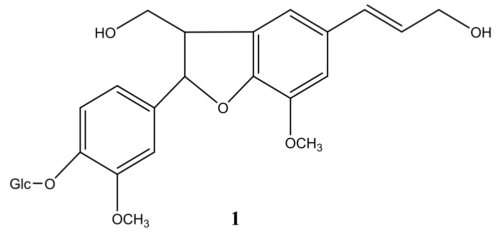
Various types of polymers have been utilized in many delivery systems as they can efficiently deliver the drugs and therapeutic agents to the target site in a controllable manner, and accordingly increase the therapeutic benefit, while minimizing the side effects **[10]**.

In this article we have studied the phytochemical constituents of flax seeds and the biological activities of their extract fractions. The antimicrobial activities of the extract fractions were also assessed. Additionally, the bioactive butanol subfraction, containing lignans, was incorporated into pluronic nano-micelles and its controlled release profile was investigated.

2. RESULTS AND DISCUSSION

2.1 Phytochemical evaluation

The chromatographic separation of the dried aerial part extracts of Linum usitatissimum afforded 8–5' neolignan (dehydrodiconiferyl alcohol-4-β-D-glucoside **1**). The identity of which was established by comparing its 1H NMR data with the reported previously by 8–5' neolignan (dehydrodiconiferyl alcohol-4-β-D-glucoside **[11]**.



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In addition, 60 compounds from acetogenins, terpenoids, steroids, and others were identified by the GC/MS (Table 4) from fractions of hexane Lu1, Lu3a and methylene chloride Lu2, Lu3b by comparing the MS spectra with those in NIST library. A sample from hexane fraction Lu1 afforded 24 compounds, representing 95.29% from the sample, with 9,12,15-octadecatrienoic acid (80.97%), hexadecanoic acid (10.69%) and selegiline (0.56%) being the major components. Methylene chloride fraction Lu2 afforded 24 compounds, representing 85.34 % from the sample, with methyl (Z,Z,Z)9,12,15-octadecatrienoate (32.01%), butyl 9,12,15-octadecatrienoate (16.29%) and methyl 9-cis,11-trans-octadecadienoate (7.69%) being the major components. Hexane extract Lu3a afforded 19 compounds, representing 75.41 % from the sample, with methyl palmitate (38.64%), methyl (Z,Z)7,10- methyloctadecadienoate (9.91%) and methyl (Z,Z)8,11-octadecadienoate (9.85%) being the major components. Methylene chloride extract Lu3b afforded 14 compounds, representing 4.96% from the sample, with tetracosane (0.81%), eicosane (0.61%), and hexacosane (0.54%) being the major components (Table 4).

2.2 Biological applications

2.2.1 Antimicrobial activity assessment

In our current study, the antimicrobial potentials of hexane Lu1, methylene chloride Lu2, and butanol Lu3d extracts of *L. usitatissimum* were examined by disc diffusion assay method, using pathogenic microbial species; *Staphylococcus aureus*, representing Gram positive bacteria, *Escherichia coli*, representing Gram negative bacteria, and *C. albicans*, representing fungi (Table 1).

The activity index of hexane extract (Lu1), methylene chloride extract (Lu2) and butanol extract (Lu3d) against *E. coli* were comparable to *ampicillin* (29.2%, 37.5% and 66.7, respectively).

The activity index of hexane extract (Lu1), methylene chloride extract (Lu2) and butanol extract (Lu3d) against *S. aureus* were comparable to *ampicillin* (45.4%, 36.4% and 63.6%, respectively). The activity index of hexane extract (Lu1), methylene chloride extract (Lu2) and butanol extract (Lu3d) against *C. albicans* were comparable to colitrimazole (26.9%, 46.1% and 73.1%, respectively) (Table 1). Shivani and his coauthors, 2013 reported the antimicrobial inhibitory effect of leaves and flowers methanolic extracts against six strains **[12].**

Table 1: The inhibition zone in mm and activity index% of extracts of *L. usitatissimum* compared to standard antibiotics

Fraction	*E. coli* (mg/ml)		*S. aureus* (mg/ml)		*C. albicans* (mg/ml)	
	Diameter of inhibition zone (in mm)	% Activity index	Diameter of inhibition zone (in mm)	% Activity index	Diameter of inhibition zone (in mm)	% Activity index
Hexane	7	29.2	10	45.4	7	26.9
Methylene chloride	9	37.5	8	36.4	12	46.1
Butanol	16	66.7	14	63.6	19	73.1
Ampicillin	24	100	22	100	NA	----
Colitrimazole	NA	----	NA	----	26	100

2.2.2 Antioxidant activity assessment

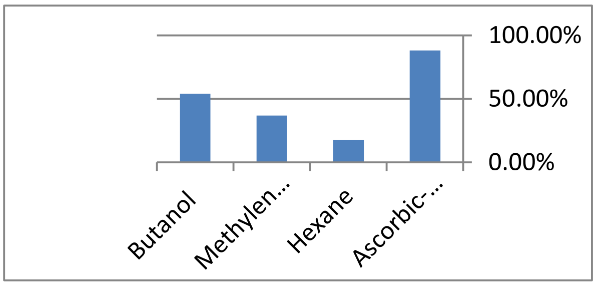
The antioxidant activity of hexane, methylene chloride and butanol fractions, was assessed using ABTS method **[13]**. A control experiment and another one using ascorbic acid as a reference antioxidant material were conducted. Table 2 showed that the butanol subfraction had inhibited (54%) of the free radicals, followed by methylene chloride fraction (36.8%). The hexane fraction had inhibited (17.6%) of the free radicals, respectively.

The free radicals of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) is used for detection of the antioxidant activity of the plant extracts**.** Butanol has the highest scavenging activity. The scavenging effect of the extracts and standard on the ABTS radical decreased in the following order: ascorbic acid > butanol fraction > methylene chloride fraction > hexane fraction. (Table 2 and Fig. 1), respectively.

Table 2: The antioxidant activity of the extracts of *L. usitatissimum* by ABTS method.

Fractions	**Absorbance of samples**	**% inhibition**
Control of ABTS	0.505	0%
Ascorbic-acid	0.060	88.1%
Hexane	0.416	17.6%
Methylene chloride	0.319	36.8%
Butanol	0.232	54.0%

Fig. 1: The antioxidant activity of the extracts of *L.*



usitatissimum by ABTS method.

2.2.3 Cytotoxic assessment

In Egypt, liver cancer is the second cause of deaths from cancer after breast cancer and it is the third frequent occurring cancer after bladder and breast cancer and hepatocellular carcinoma (HCC) is a major health problem **[14].** The natural extracts have a vital role on cancer chemoprevention and chemotherapy **[15].**

The butanol extract and methylene chloride extract were "moderate", hexane extract was "weak" against HepG2 cell line **[16]**.

The results (Table 3) indicated that the *in vitro* cytotoxicity against MCF-7, IC_{50} (µg/ml), of the butanol extract was "strong", methylene chloride extract and hexane extract were "weak".

Table 3: Cytotoxic activity assessment of *L. usitatissimum* extracts against human tumor cells HePG2 and MCF-7.

Fractions	In vitro Cytotoxicity IC50 (µg/ml)	
	MCF-7	HePG2
5-FU	4.7±0.11	6.6±0.24
Hexane	63.0±3.03	76.7±2.75
Methylene chloride	55.8±2.84	47.5±1.96
Butanol	13.5±0.85	23.2±1.10

IC50 (µg/ml): 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic)

2.3 Preparation of Lu 3d-loaded pluronic nanoparticles

The Lu 3d-loaded pluronic nanoparticles were prepared using nanoprecipitation technique through a modified procedure. This preparation technique is appropriate for encapsulation of hydrophobic compounds such as the subfraction Lu 3d obtained in the current study. Also, the encapsulation of these water-insoluble compounds into nano-sized particles enhances their dissolution and consequently improves their bioavailability. It was found that their limited aqueous solubility has been enhanced upon their nanoformulation. Nanoformulation of Lu 3d has also led to a stable colloidal dispersion with strong green color, indicating their homogenous distribution in the aqueous medium at a significantly higher concentration than that obtained using other solvents.

2.4 Particle size, zeta potential and surface morphology

The average diameters of the prepared plain and Lu 3d-loaded pluronic nanoparticles were found to be 207 ± 12 and 225 ± 18 nm, respectively as determined by DLS (Figure 2). As can also be noted, the particle size of plain nanoparticles is relatively less than that of Lu 3d-loaded nanoparticles. The charges on the developed plain and Lu 3d-loaded nanoparticles were found negative with the values of -11 and -17, respectively as determined from the zeta potential measurements (Figure 3). The size of the prepared nanoparticles was also estimated from the transmission electron microscopy, TEM (Figure 4). With the aid of TEM particle analysis software (NanoScope Analysis, Veeco Co, USA), the average diameter of these plain and Lu 3d-loaded pluronic nanoparticles was found to be 18 ± 2.6 nm and 48 ± 2.9 nm, respectively.

The TEM micrographs of plain pluronic nanoparticles and Lu 3d-loaded nanoparticles are illustrated in Figure 4. From the figure, it can be noted that all the developed plain and Lu 3d-loaded nanoparticles are spherical with almost smooth surfaces.

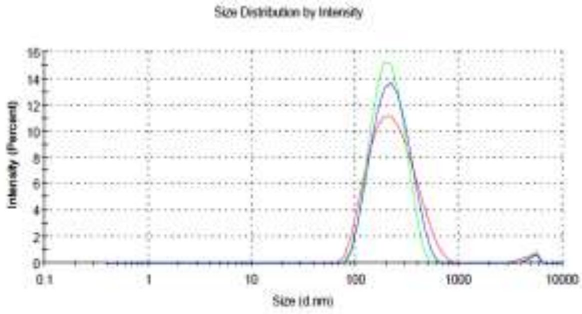


Figure 2. The mean particle size of the developed pluronic nanoparticles.

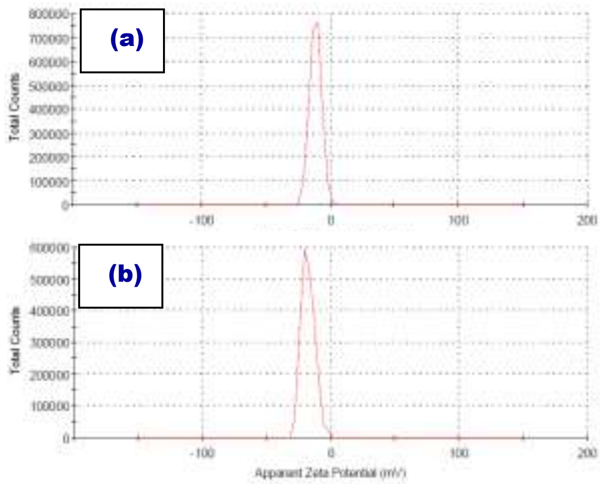
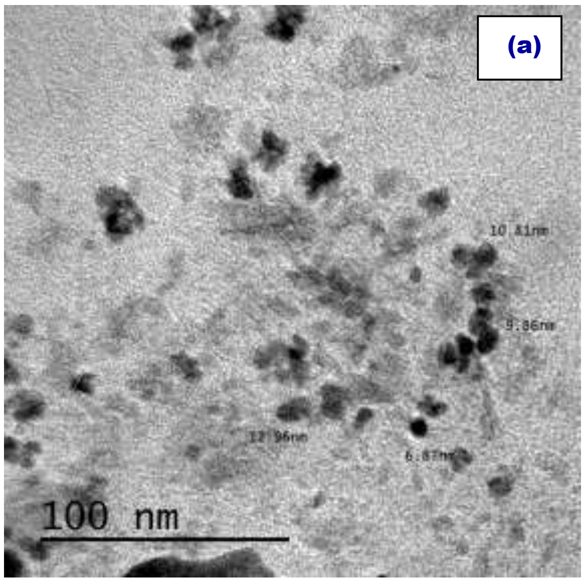


Figure 3. Zeta potential values of (a) plain pluronic nanoparticles, and (b) Lu 3d-loaded pluronic nanoparticles.



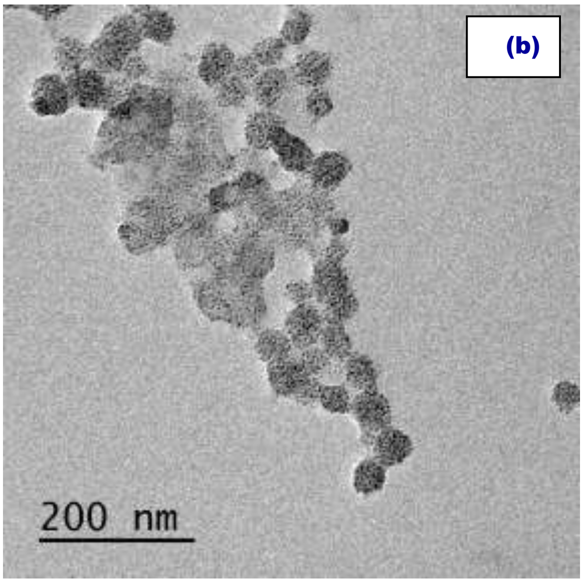


Figure 4. Transmission electron micrographs of (a) plain pluronic nanoparticles, and (b) Lu 3d-loaded pluronic nanoparticles.

2.5 *In-vitro* biodegradation study

An *in-vitro* biodegradation study of the Lu 3d-loaded pluronic nanoparticles was performed in PBS, pH 7.4 in presence of lysozyme. The weight loss percentage of the nanoparticles as a function of time was determined and taken as a measure of degradation. Figure 5 demonstrates the degradation pattern of the developed nanoparticles. As apparent from the figure, the nanoparticles attained higher degradation rates in presence of lysozyme. For instance, the percentage of degraded weights (W_d%) in presence of the enzyme was almost 89.4% after 2 days.

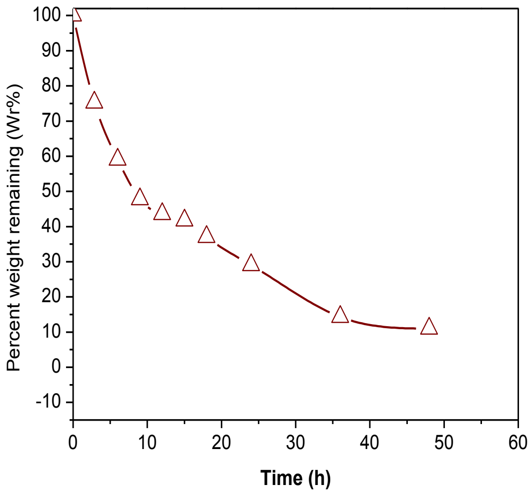


Figure 5. Degradation profile of the developed Lu 3d-loaded pluronic nanoparticles at 37°C in PBS (pH 7.4). The values are average of three triplicates.

2.6 *In-vitro* cumulative release studies

The entrapment efficiency (EE%) of the loaded Lu 3d in the developed pluronic nanoparticles was determined and found to be in the range 88.9 ± 1 to 90.2 ± 2.

The cumulative release profile of the loaded Lu 3d from the pluronic nanoparticles at 37°C in PBS of pH 7.4 is shown in Figure 6. As apparent from the figure, the percent of the Lu 3d-loaded pluronic nanoparticles released increased gradually with time until attaining almost a constant value after about 36 hours.

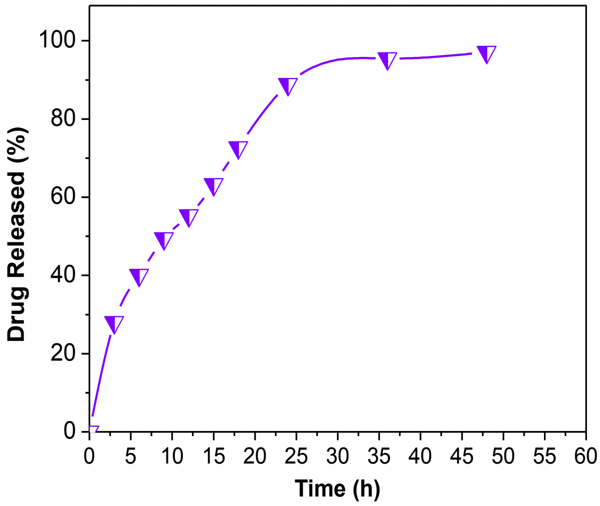


Figure 6. Release study of the loaded Lu 3d from the pluronic nanoparticles at 37°C in PBS, pH 7.4. The values are average of three triplicates.

Table 4: MS data of compounds identified by GC/MS analyses (m/z [identity] (rel.int. %))

Compound Name	MS Data: m/z [identity] (rel. abound.%)
dodecane	170 $[M]^+$ (6.66), 127 $[C_9H_{19}]^+$ (3.33), 112 $[C_8H_{16}]^+$ (4), 98 $[C_7H_{14}]^+$ (6.66), 85 $[C_6H_{13}]^+$ (36.66), 71 $[C_5H_{11}]^+$ (56.66), 57 $[C_4H_9]^+$ (100).
tetradecane	198 $[M]^+$ (5),169 $[C_{12}H_{25}]^+$ (1.66), 155 $[C_{11}H_{23}]^+$ (2.66),141 $[C_{10}H_{21}]^+$ (3.33), 126 $[C_9H_{18}]^+$ (3.33),112 $[C_8H_{16}]^+$ (6.66), 99 $[C_7H_{15}]^+$ (10), 85 $[C_6H_{13}]^+$ (46.66), 71 $[C_5H_{11}]^+$ (70), 57 $[C_4H_9]^+$ (100).
hexadecane	226 $[M]^+$ (5.55), 183 $[C_{13}H_{27}]^+$ (1.11), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (3.33), 141 $[C_{10}H_{21}]^+$ (4.44), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (10), 99 $[C_7H_{15}]^+$ (16.66), 85 $[C_6H_{13}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100).
heptadecane	240 $[M]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (2.22), 183 $[C_{13}H_{27}]^+$ (2.44), 169 $[C_{12}H_{25}]^+$ (2.22), 155 $[C_{11}H_{23}]^+$ (2.22), 141 $[C_{10}H_{21}]^+$ (4.44), 127 $[C_9H_{19}]^+$ (4.44), 111 $[C_8H_{15}]^+$ (14.44), 97 $[C_7H_{13}]^+$ (67.77), 85 $[C_6H_{13}]^+$ (28.88), 71 $[C_5H_{11}]^+$ (44.44), 57 $[C_4H_9]^+$ (100).
octadecane	254 $[M]^+$ (3.33), 225 $[C_{16}H_{33}]^+$ (1.11), 211 $[C_{15}H_{31}]^+$ (1.11), 197 $[C_{14}H_{29}]^+$ (2.22), 183 $[C_{13}H_{27}]^+$ (2.44), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (2.44), 141 $[C_{10}H_{21}]^+$ (4.44), 127 $[C_9H_{19}]^+$ (6.66), 113 $[C_8H_{17}]^+$ (11.11), 99 $[C_7H_{15}]^+$ (18.88), 85 $[C_6H_{13}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (68.88), 57 $[C_4H_9]^+$ (100),
eicosane	282 $[M]^+$ (3.33), 241 $[C_{17}H_{37}]$ (3), 224 $[C_{16}H_{32}]^+$ (3.33), 211 $[C_{15}H_{31}]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (3.33), 183 $[C_{13}H_{27}]^+$ (3.33), 169 $[C_{12}H_{25}]^+$ (3.66), 141 $[C_{10}H_{21}]^+$ (6.66), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.33), 99 $[C_7H_{15}]^+$ (21.66), 85 $[C_6H_{13}]^+$ (60), 71 $[C_5H_{11}]^+$ (75), 57 $[C_4H_9]^+$ (100).
heneicosane	296 $[M]^+$ (7.69), 281 $[C_{20}H_{41}]^+$ (2.20), 267 $[C_{19}H_{39}]^+$ (1.98), 253 $[C_{18}H_{37}]^+$ (2.20), 239 $[C_{17}H_{35}]^+$ (4.40), 225 $[C_{16}H_{33}]^+$ (4.40), 211 $[C_{15}H_{31}]^+$ (3.30), 197 $[C_{14}H_{29}]^+$ (5.49), 183 $[C_{13}H_{27}]^+$ (6.59), 169 $[C_{12}H_{25}]^+$ (6.59), 155 $[C_{11}H_{23}]^+$ (9.01), 141 $[C_{10}H_{21}]^+$ (9.89), 125 $[C_9H_{17}]^+$ (17.58), 111 $[C_8H_{15}]^+$ (29.67), 97 $[C_7H_{13}]^+$ (46.15), 85 $[C_6H_{13}]^+$ (59.34), 71 $[C_5H_{11}]^+$ (74.95), 57 $[C_4H_9]^+$ (100).
docosane	310 $[M]^+$ (10.99), 281 $[C_{20}H_{41}]^+$ (2.20), 267 $[C_{19}H_{39}]^+$ (2.42), 253 $[C_{18}H_{37}]^+$ (3.30), 239 $[C_{17}H_{35}]^+$ (2.42), 225 $[C_{16}H_{33}]^+$ (4.40), 211 $[C_{15}H_{31}]^+$ (4.40), 197 $[C_{14}H_{29}]^+$ (4.40), 183 $[C_{13}H_{27}]^+$ (6.59), 169 $[C_{12}H_{25}]^+$ (6.59), 155 $[C_{11}H_{23}]^+$ (8.79), 141 $[C_{10}H_{21}]^+$ (10.99), 125 $[C_9H_{17}]^+$ (13.19), 111 $[C_8H_{15}]^+$ (22.20), 97 $[C_7H_{13}]^+$ (28.79), 85 $[C_6H_{13}]^+$ (61.54), 71 $[C_5H_{11}]^+$ (80.22), 57 $[C_4H_9]^+$ (100).
tricosane	324 $[M]^+$ (8.79), 295 $[C_{21}H_{43}]^+$ (2.20), 281 $[C_{20}H_{41}]^+$ (4.18), 267 $[C_{19}H_{39}]^+$ (3.30), 253 $[C_{18}H_{37}]^+$ (3.30), 239 $[C_{17}H_{35}]^+$ (3.30), 225 $[C_{16}H_{33}]^+$ (4.62), 211 $[C_{15}H_{31}]^+$ (4.84), 197 $[C_{14}H_{29}]^+$ (5.49), 183 $[C_{13}H_{27}]^+$ (6.59), 169 $[C_{12}H_{25}]^+$ (6.59), 155 $[C_{11}H_{23}]^+$ (10.77), 141 $[C_{10}H_{21}]^+$ (9.89), 125 $[C_9H_{17}]^+$ (17.58), 111 $[C_8H_{15}]^+$ (29.67), 97 $[C_7H_{13}]^+$ (44.18), 85 $[C_6H_{13}]^+$ (62.64), 71$[C_5H_{11}]^+$ (81.10), 57 $[C_4H_9]^+$ (100).
tetracosane	338 $[M]^+$ (1.11), 310 $[C_{22}H_{46}]$ (2.22), 281 $[C_{20}H_{41}]^+$ (2.22), 267 $[C_{19}H_{39}]^+$ (2.22), 253 $[C_{18}H_{37}]^+$ (1.11), 239 $[C_{17}H_{35}]^+$ (2), 225 $[C_{16}H_{33}]^+$ (2.22), 211 $[C_{15}H_{31}]^+$ (2.22), 197 $[C_{14}H_{29}]^+$ (2.22), 183 $[C_{13}H_{27}]^+$ (3.33), 169 $[C_{12}H_{25}]^+$ (2.44), 155 $[C_{11}H_{23}]^+$ (5.55), 141 $[C_{10}H_{21}]^+$ (7.77), 127 $[C_9H_{19}]^+$ (8.88), 113 $[C_8H_{17}]^+$ (13.33), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (48.88), 71 $[C_5H_{11}]^+$ (71.11), 57 $[C_4H_9]^+$ (100).
pentacosane	352 $[M]^+$ (2.22), 337 $[C_{24}H_{49}]^+$ (1.11), 295 $[C_{21}H_{43}]^+$ (0.66), 281 $[C_{20}H_{41}]^+$ (6.44), 267 $[C_{19}H_{39}]^+$ (3.33), 253 $[C_{18}H_{37}]^+$ (3.33), 239 $[C_{17}H_{35}]^+$ (2.22), 225 $[C_{16}H_{33}]^+$ (2.22), 211 $[C_{15}H_{31}]^+$ (2.22), 197 $[C_{14}H_{29}]^+$ (4.44), 183 $[C_{13}H_{27}]^+$ (3.33), 169n $[C_{12}H_{25}]^+$ (4.44), 155 $[C_{11}H_{23}]^+$ (6.44), 141 $[C_{10}H_{21}]^+$ (8.88), 129 $[C_9H_{21}]^+$ (13.55), 111 $[C_8H_{15}]^+$ (17.55), 97 $[C_7H_{13}]^+$ (26.66), 85 $[C_6H_{13}]^+$ (54.44), 71 $[C_5H_{11}]^+$ (73.33), 57 $[C_4H_9]^+$ (100).
hexacosane	366$[M]^+$(2.44),309$[C_{22}H_{45}]^+$(1.11),295$[C_{21}H_{43}]^+$(1.11),281$[C_{20}H_{41}]^+$(5.55),267$[C_{19}H_{39}]^+$(3.33),253$[C_{18}H_{37}]^+$(3.33),239$[C_{17}H_{35}]^+$(2.22),225$[C_{16}H_{33}]^+$(2),211$[C_{15}H_{31}]^+$(2.44),197$[C_{14}H_{29}]^+$(3.33),183$[C_{13}H_{27}]^+$(3.33),169$[C_{12}H_{25}]^+$(4.44),155$[C_{11}H_{23}]^+$(6.66),141$[C_{10}H_{21}]^+$(7.77),129$[C_9H_{21}]^+$(10),113$[C_8H_{17}]^+$(13.33),97$[C_7H_{13}]^+$(21.11),85$[C_6H_{13}]^+$(48.88),71$[C_5H_{11}]^+$(62.22),57$[C_4H_9]^+$(100),
heptacosane	380 $[M]^+$ (2.22), 337 $[C_{24}H_{49}]^+$ (1.11), 309 $[C_{22}H_{45}]^+$ (1.11), 295 $[C_{21}H_{43}]^+$ (2), 281 $[C_{20}H_{41}]^+$ (8.88), 267 $[C_{19}H_{39}]^+$ (4.44), 253 $[C_{18}H_{37}]^+$ (4.44), 239 $[C_{17}H_{35}]^+$ (2.22), 225 $[C_{16}H_{33}]^+$ (2.66), 211 $[C_{15}H_{31}]^+$ (2.66), 197 $[C_{14}H_{29}]^+$ (4.44), 183 $[C_{13}H_{27}]^+$ (2.66), 169 $[C_{12}H_{25}]^+$ (6.66), 155 $[C_{11}H_{23}]^+$ (7.77), 141 $[C_{10}H_{21}]^+$ (8.88), 129 $[C_9H_{21}]^+$ (14.44), 113 $[C_8H_{17}]^+$ (15.55), 97 $[C_7H_{13}]^+$ (27.77), 85 $[C_6H_{13}]^+$ (54.44), 71 $[C_5H_{11}]^+$ (75.55), 57 $[C_4H_9]^+$ (100).

octacosane	394 $[M]^+$ (1.66), 337 $[C_{24}H_{49}]^+$ (0.66), 323 $[C_{23}H_{47}]^+$ (0.66), 309 $[C_{22}H_{45}]^+$ (1.66), 295 $[C_{21}H_{43}]^+$ (1.66), 281 $[C_{20}H_{41}]^+$ (3.66), 267 $[C_{19}H_{39}]^+$ (3), 253 $[C_{18}H_{37}]^+$ (3), 239 $[C_{17}H_{35}]^+$ (3.33), 225 $[C_{16}H_{33}]^+$ (1.66), 211 $[C_{15}H_{31}]^+$ (3.33), 197 $[C_{14}H_{29}]^+$ (3.33), 183 $[C_{13}H_{27}]^+$ (3.33), 169 $[C_{12}H_{25}]^+$ (3.33), 141 $[C_{10}H_{21}]^+$ (6.66), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.33), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (56.66), 71 $[C_5H_{11}]^+$ (76.66), 57 $[C_4H_9]^+$ (100).
nonacosane	408 $[M]^+$ (1.66), 379 $[C_{27}H_{55}]^+$ (0.33), 351 $[C_{25}H_{51}]^+$ (0.33), 309 $[C_{22}H_{45}]^+$ (0.33), 365 $[C_{26}H_{53}]^+$ (0.33), 323 $[C_{23}H_{47}]^+$ (0.66), 295 $[C_{21}H_{43}]^+$ (1.66), 281 $[C_{20}H_{41}]^+$ (5), 267 $[C_{19}H_{39}]^+$ (1.33), 253 $[C_{18}H_{37}]^+$ (3), 239 $[C_{17}H_{35}]^+$ (1.66), 225 $[C_{16}H_{33}]^+$ (2.66), 183 $[C_{13}H_{27}]^+$ (3.33), 169 $[C_{12}H_{25}]^+$ (3.33), 155 $[C_{11}H_{23}]^+$ (5), 141 $[C_{10}H_{21}]^+$ (8.33), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (13.33), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (56.66), 71 $[C_5H_{11}]^+$ (76.66), 57 $[C_4H_9]^+$ (100).
triacontane	422 $[M]^+$ (1.66), 365 $[C_{26}H_{53}]^+$ (0.33), 323 $[C_{23}H_{47}]^+$ (0.66), 309 $[C_{22}H_{45}]^+$ (1.66), 295 $[C_{21}H_{43}]^+$ (1.66), 281 $[C_{20}H_{41}]^+$ (6.66), 267 $[C_{19}H_{39}]^+$ (1.33), 253 $[C_{18}H_{37}]^+$ (3.33), 239 $[C_{17}H_{35}]^+$ (3.33), 225 $[C_{16}H_{33}]^+$ (1.66), 211 $[C_{15}H_{31}]^+$ (3),197 $[C_{14}H_{29}]^+$ (3.33), 183 $[C_{13}H_{27}]^+$ (3.33), 169 $[C_{12}H_{25}]^+$ (3.66), 155 $[C_{11}H_{23}]^+$ (5), 141 $[C_{10}H_{21}]^+$ (6.66), 127 $[C_9H_{19}]^+$ (10), 113 $[C_8H_{17}]^+$ (11.66), 99 $[C_7H_{15}]^+$ (20), 85 $[C_6H_{13}]^+$ (55), 71 $[C_5H_{11}]^+$ (76.66), 57 $[C_4H_9]^+$ (100).
hentriacontane	436 $[M]^+$ (1.11), 405 $[C_{29}H_{57}]^+$ (4.44), 295 $[C_{21}H_{43}]^+$ (2), 281 $[C_{20}H_{41}]^+$ (27.78), 267 $[C_{19}H_{39}]^+$ (11.11), 253 $[C_{18}H_{37}]^+$ (13.56), 97 $[C_7H_{13}]^+$ (22.67), 85 $[C_6H_{13}]^+$ (31.11), 73 $[C_5H_{13}]^+$ (100), 57 $[C_4H_9]^+$ (66.67).
dotriacontane	450 $[M]^+$ (4.62), 405 (8.79), 379 (3.30), 355 (15.60), 337 $[C_{24}H_{49}]^+$ (3.30), 327 (15.38), 323 $[C_{23}H_{47}]^+$ (2.20), 309 $[C_{22}H_{45}]^+$ (2.20), 295 $[C_{21}H_{43}]^+$ (4.40), 281 $[C_{20}H_{41}]^+$ (50.55), 267 $[C_{19}H_{39}]^+$ (18.68), 253 $[C_{18}H_{37}]^+$ (27.47), 239 $[C_{17}H_{35}]^+$ (8.79), 225 $[C_{16}H_{33}]^+$ (4.40), 211 $[C_{15}H_{31}]^+$ (6.81), 197 $[C_{14}H_{29}]^+$ (10.99), 183 $[C_{13}H_{27}]^+$ (10.99), 169 $[C_{12}H_{25}]^+$ (6.81), 155 $[C_{11}H_{23}]^+$ (10.99), 141 $[C_{10}H_{21}]^+$ (13.19), 135 (30.77), 127 $[C_9H_{19}]^+$ (15.38), 111 $[C_8H_{15}]^+$ (26.37), 97 $[C_7H_{13}]^+$ (46.15), 85$[C_6H_{13}]^+$ (60.44), 71 $[C_5H_{11}]^+$ (82.42), 57 $[C_4H_9]^+$ (100).
2- methyleicosane	296 $[M]^+$ (10.99), 281 $[C_{20}H_{41}]^+$ (20.88), 267 $[C_{19}H_{39}]^+$ (6.59), 253 $[C_{18}H_{37}]^+$ (6.37), 239 $[C_{17}H_{35}]^+$ (5.49), 225 $[C_{16}H_{33}]^+$ (4.62), 211 $[C_{15}H_{31}]^+$ (4.40), 197 $[C_{14}H_{29}]^+$ (6.59), 183 $[C_{13}H_{27}]^+$ (7.69), 169 $[C_{12}H_{25}]^+$ (12.09), 155 $[C_{11}H_{23}]^+$ (11.21), 141 $[C_{10}H_{21}]^+$ (13.19), 125 $[C_9H_{17}]^+$ (23.08), 111 $[C_8H_{15}]^+$ (39.56), 97 $[C_7H_{13}]^+$ (51.65), 83 $[C_6H_{11}]^+$ (79.12), 71 $[C_5H_{11}]^+$ (68.13), 57 $[C_4H_9]^+$ (100).
cetene	224 $[M]^+$ (2.44), 196 $[C_{14}H_{28}]^+$ (2.22), 181 $[C_{13}H_{25}]^+$ (1.11), 168 $[C_{12}H_{24}]$ (2), 153 $[C_{11}H_{21}]^+$ (2.44), 139 $[C_{10}H_{19}]^+$ (4.66), 125 $[C_9H_{17}]^+$ (15.55), 111 $[C_8H_{15}]^+$ (35.55), 97 $[C_7H_{13}]^+$ (77.77), 83 $[C_6H_{11}]^+$ (86.66), 69 $[C_5H_9]^+$ (84.44), 55 $[C_4H_7]^+$ (100).
1-octadecene	252 $[M]^+$ (2.22), 224 $[C_{16}H_{32}]$ (1.11), 195 $[C_{14}H_{27}]^+$ (0.44), 181 $[C_{13}H_{25}]^+$ (2), 167 $[C_{12}H_{23}]^+$ (2.22), 153 $[C_{11}H_{21}]^+$ (4.44), 139 $[C_{10}H_{19}]^+$ (6.88), 125 $[C_9H_{17}]^+$ (17.77), 111 $[C_8H_{15}]^+$ (42.22), 97 $[C_7H_{13}]^+$ (82.11), 83 $[C_6H_{11}]^+$ (94.44), 69 $[C_5H_9]^+$ (78), 55 $[C_4H_7]^+$ (100).
2,4-heptadienal	110 $[M]^+$ (21.66), 95 $[C_6H_7O]^+$ (5), 81 $[C_5H_5O]^+$ (100), 67 (13.33), 53 $[C_3HO]^+$ (18.33).
2-decenal	154 $[M]^+$ (0.67), 110 $[C_7H_{10}O]^+$ (16.67), 98 $[C_6H_{10}O]^+$ (30), 83 $[C_5H_7O]^+$ (66.67), 70 $[C_4H_6O]^+$ (100), 55 $[C_3H_3O]^+$ (86.67).
9,17-octadecadienal	264 $[M]^+$ (3.33), 235 $[C_{17}H_{31}]^+$ (1.66), 221 $[C_{16}H_{29}]^+$ (6.66), 207 $[C_{15}H_{27}]^+$ (3.33), 193 $[C_{14}H_{25}]^+$ (1.66), 179 $[C_{13}H_{23}]^+$ (1), 165 $[C_{12}H_{21}]^+$ (3.33), 151 $[C_{11}H_{19}]^+$ (6.66), 137 $[C_{10}H_{17}]^+$ (16.66), 129 $[C_8H_{17}O]^+$ (26.66), 111 $[C_8H_{15}]^+$ (20), 95 $[C_7H_{11}]^+$ (53.33), 85 $[C_5H_9O]^+$ (50), 67 $[C_5H_7]^+$ (73.33), 55 $[C_4H_7]^+$ (100).
9,12,15-octadecatrien-1-ol	264 $[M]^+$ (5), 249 $[C_{17}H_{29}O]^+$ (1.66), 235 $[C_{16}H_{27}O]^+$ (1.66), 195 $[C_{13}H_{23}O]^+$ (1.66), 169 $[C_{11}H_{21}O]^+$ (5), 135 $[C_{10}H_{15}]^+$ (13.33), 129 $[C_8H_{17}O]^+$ (16.66), 115 $[C_7H_{15}O]^+$ (8.33), 109 $[C_8H_{13}]^+$ (38.33), 95 $[C_7H_{11}]^+$ (65), 79 (100), 69 $[C_5H_9]^+$ (83.33), 55 $[C_4H_7]^+$ (78.33).
pentadecanoic acid	242 $[M]^+$ (48.33), 227 $[C_{14}H_{27}O_2]^+$ (3.33), 213 $[C_{13}H_{25}O_2]^+$ (20), 199 $[C_{12}H_{23}O_2]^+$ (30), 185 $[C_{11}H_{21}O_2]^+$ (21.66), 171 $[C_{10}H_{19}O_2]^+$ (15), 157 $[C_9H_{17}O_2]^+$ (13.33), 143 $[C_8H_{15}O_2]^+$ (23.33), 129 $[C_7H_{13}O_2]^+$ (50), 115 $[C_6H_{11}O_2]^+$ (20), 101 $[C_5H_9O_2]^+$ (10), 87 $[C_4H_7O_2]^+$ (21.66), 73 $[C_3H_5O_2]^+$ (100), 60 $[C_2H_4O_2]$ (73.33).
hexadecanoic acid	256 $[M]^+$ (81.66), 227 $[C_{14}H_{27}O_2]^+$ (16.66), 213 $[C_{13}H_{25}O_2]^+$ (61.66), 199 $[C_{12}H_{23}O_2]^+$ (16.66), 185 $[C_{11}H_{21}O_2]^+$ (33.33), 171 $[C_{10}H_{19}O_2]^+$ (30), 157 $[C_9H_{17}O_2]^+$ (33.33), 143 $[C_8H_{15}O_2]^+$ (13.33), 129 $[C_7H_{13}O_2]^+$ (73.33), 115 $[C_6H_{11}O_2]^+$ (23.33), 101 $[C_5H_9O_2]^+$ (13.33), 87 $[C_4H_7O_2]^+$ (23.33), 73 $[C_3H_5O_2]^+$ (100), 60 $[C_2H_4O_2]$ (68.33).
heptadecanoic acid	270 $[M]^+$ (13.33), 241 $[C_{15}H_{29}O_2]^+$ (3.33), 227 $[C_{14}H_{27}O_2]^+$ (15), 213 $[C_{13}H_{25}O_2]^+$ (15), 199 $[C_{12}H_{23}O_2]^+$ (6.66), 185 $[C_{11}H_{21}O_2]^+$ (21.66), 171 $[C_{10}H_{19}O_2]^+$ (16.66), 157 $[C_9H_{17}O_2]^+$ (10), 143 $[C_8H_{15}O_2]^+$ (7), 129 $[C_7H_{13}O_2]^+$ (43.33), 111 $[C_8H_{15}]^+$ (16.66), 101 $[C_5H_9O_2]^+$ (10), 95 (58.33), 73 $[C_3H_5O_2]^+$ (80), 55 $[C_4H_7]^+$ (100).

(Z,Z)9,12-octadecadienoic acid	280[M]$^+$(5),263[$C_{18}H_{31}O$]$^+$(6.66),237[$C_{15}H_{25}O_2$]$^+$(1.66),223[$C_{14}H_{23}O_2$]$^+$(6.66),209[$C_{13}H_{21}O_2$]$^+$(3.33),183[$C_{11}H_{21}O_2$](6.66),165[$C_{12}H_{21}$]$^+$(3.33),149[$C_{11}H_{17}$]$^+$(10),143[$C_8H_{15}O_2$]$^+$(5),137[$C_{10}H_{17}$]$^+$(11.66),129[$C_7H_{13}O_2$]$^+$(20),115[$C_6H_{11}O_2$]$^+$(10),110[C_8H_{14}]$^+$(18.33),95[C_7H_{11}]$^+$(75),55[C_4H_7]$^+$(100),
(E,E)9,12-octadecadienoic acid	280 [M]$^+$ (6.66), 265 [$C_{17}H_{29}O_2$]$^+$ (5), 235 [$C_{17}H_{31}$]$^+$ (1.66), 222 [$C_{14}H_{22}O_2$]$^+$ (10), 209 [$C_{13}H_{21}O_2$]$^+$ (3.33), 185 [$C_{11}H_{21}O_2$] (3.33), 169 [$C_{10}H_{17}O_2$]$^+$ (1.66), 165 [$C_{12}H_{21}$]$^+$ (1.66), 149 [$C_{11}H_{17}$]$^+$ (10), 143 [$C_8H_{15}O_2$]$^+$ (3), 135 [$C_{10}H_{15}$]$^+$ (13.33), 129 [$C_7H_{13}O_2$]$^+$ (10),97 [C_7H_{13}]$^+$ (66.66), 79 (100), 55[C_4H_7]$^+$ (70).
methyl (Z,Z,Z)9,12,15-octadecatrienoate	278 [M]$^+$ (6.66), 263 [$C_{17}H_{27}O_2$]$^+$ (5), 249 [$C_{16}H_{25}O_2$]$^+$ (3.33), 222 [$C_{14}H_{22}O_2$]$^+$ (13.33), 185 [$C_{11}H_{21}O_2$] (3), 163 [$C_{12}H_{19}$]$^+$ (3.33), 149 [$C_{11}H_{17}$]$^+$ (10), 135 [$C_{10}H_{15}$]$^+$ (13.33), 108 [C_8H_{12}]$^+$ (41.66), 95 [C_7H_{11}]$^+$ (53.33), 79 (100), 69 [C_5H_9]$^+$ (60), 55 [C_4H_7]$^+$ (43.33).
methyl tetradecanoate	242 [M]$^+$ (6.88), 211 [$C_{14}H_{27}O$]$^+$ (4.44), 199 [$C_{12}H_{23}O_2$]$^+$ (11.11), 185 [$C_{11}H_{21}O_2$]$^+$ (4.44), 171 [$C_{10}H_{19}O_2$]$^+$ (2.22), 157 [$C_9H_{17}O_2$]$^+$ (4.44), 143 [$C_8H_{15}O_2$]$^+$ (20), 129 [$C_7H_{13}O_2$]$^+$ (6.66), 115 [$C_6H_{11}O_2$]$^+$ (2.22), 101 [$C_5H_9O_2$]$^+$ (6.88), 87 [$C_4H_7O_2$]$^+$ (62.44), 74 [$C_3H_6O_2$] (100), 55 [C_4H_7]$^+$ (26.88).
methyl pentadecanoate	256 [M]$^+$ (8.88), 227 [$C_{14}H_{27}O_2$]$^+$ (4.44), 213 [$C_{13}H_{25}O_2$]$^+$ (7.77), 199 [$C_{12}H_{23}O_2$]$^+$ (4.44), 185 [$C_{11}H_{21}O_2$]$^+$ (3.33), 171 [$C_{10}H_{19}O_2$]$^+$ (3.33), 157 [$C_9H_{17}O_2$]$^+$ (5.55), 143 [$C_8H_{15}O_2$]$^+$ (17.77), 129 [$C_7H_{13}O_2$]$^+$ (6.66), 115 [$C_6H_{11}O_2$]$^+$ (2.44), 101 [$C_5H_9O_2$]$^+$ (6.66), 87 [$C_4H_7O_2$]$^+$ (62.44), 74 [$C_3H_6O_2$] (100), 55 [C_4H_7]$^+$ (27.77).
methyl hexadecanoate	270 [M]$^+$ (11.11), 227 [$C_{14}H_{27}O_2$]$^+$ (7.77), 213 [$C_{13}H_{25}O_2$]$^+$ (2.22), 199 [$C_{12}H_{23}O_2$]$^+$ (4.44), 185 [$C_{11}H_{21}O_2$]$^+$ (5.55), 171 [$C_{10}H_{19}O_2$]$^+$ (6.66), 157 [$C_9H_{17}O_2$]$^+$ (3.33), 143 [$C_8H_{15}O_2$]$^+$ (17.77), 129 [$C_7H_{13}O_2$]$^+$ (8.88), 115 [$C_6H_{11}O_2$]$^+$ (4.44), 101 [$C_5H_9O_2$]$^+$ (6.66), 87 [$C_4H_7O_2$]$^+$ (64.44), 74 [$C_3H_6O_2$] (100), 55 [C_4H_7]$^+$ (30).
methyl octadecanoate	298 [M]$^+$ (14.44), 281 [$C_{18}H_{33}O_2$]$^+$ (1.11), 241 [$C_{17}H_{37}$] (2.44), 255 [$C_{16}H_{31}O_2$]$^+$ (8.88), 241 [$C_{15}H_{29}O_2$]$^+$ (2.44), 199 [$C_{12}H_{23}O_2$]$^+$ (8.88), 185 [$C_{11}H_{21}O_2$]$^+$ (5.55), 171 [$C_{10}H_{19}O_2$]$^+$ (2.22), 157 [$C_9H_{17}O_2$]$^+$ (4.44), 143 [$C_8H_{15}O_2$]$^+$ (22.22), 129 [$C_7H_{13}O_2$]$^+$ (13.33), 117 [$C_6H_{13}O_2$] (7.77), 101 [$C_5H_9O_2$]$^+$ (7.77), 97 [C_7H_{13}]$^+$ (14.44), 87 [$C_4H_7O_2$]$^+$ (67.77), 74 [$C_3H_6O_2$] (100), 55 [C_4H_7]$^+$ (47.77).
methyl eicosanoate	326 [M]$^+$ (13.55), 295[$C_{19}H_{35}O_2$]$^+$ (2.22), 283[$C_{18}H_{35}O_2$]$^+$ (4.66), 269 [$C_{17}H_{33}O_2$]$^+$ (1.11), 255 [$C_{16}H_{31}O_2$]$^+$ (2), 241 [$C_{15}H_{29}O_2$]$^+$ (2.22),227 [$C_{14}H_{27}O_2$]$^+$ (2.44), 213 [$C_{13}H_{25}O_2$]$^+$ (2), 199 [$C_{12}H_{23}O_2$]$^+$ (3.33), 185 [$C_{11}H_{21}O_2$]$^+$ (2.44), 171 [$C_{10}H_{19}O_2$]$^+$ (2.22), 157 [$C_9H_{17}O_2$]$^+$ (2.22), 143 [$C_8H_{15}O_2$]$^+$ (15.55), 129 [$C_7H_{13}O_2$]$^+$ (6.88), 115 [$C_6H_{11}O_2$]$^+$ (2.44), 101 [$C_5H_9O_2$]$^+$ (6.66), 97 [C_7H_{13}]$^+$ (11.55), 87 [$C_4H_7O_2$]$^+$ (65.55), 74 [$C_3H_6O_2$] (100), 55 [C_4H_7]$^+$ (40).
methyl docosanoate	354 [M]$^+$ (20), 323 [$C_{22}H_{43}O$]$^+$ (2.22), 311 [$C_{20}H_{39}O_2$]$^+$ (6.66), 297 [$C_{19}H_{37}O_2$]$^+$ (2), 283 [$C_{18}H_{35}O_2$]$^+$ (0.44), 269 [$C_{17}H_{33}O_2$]$^+$ (2.22), 255 [$C_{16}H_{31}O_2$]$^+$ (4.44), 241 [$C_{15}H_{29}O_2$]$^+$ (2.22), 227 [$C_{14}H_{27}O_2$]$^+$ (1.11), 213 [$C_{13}H_{25}O_2$]$^+$ (2), 199 [$C_{12}H_{23}O_2$]$^+$ (4.44), 185 [$C_{11}H_{21}O_2$]$^+$ (2.44), 171 [$C_{10}H_{19}O_2$]$^+$ (2), 157 [$C_9H_{17}O_2$]$^+$ (2.22), 143 [$C_8H_{15}O_2$]$^+$ (18.88), 129 [$C_7H_{13}O_2$]$^+$ (8.88), 115 [$C_6H_{11}O_2$]$^+$ (2.44), 101 [$C_5H_9O_2$]$^+$ (7.77), 97 [C_7H_{13}]$^+$ (14.44), 87 [$C_4H_7O_2$]$^+$ (78.88), 74 [$C_3H_6O_2$] (100), 55 [C_4H_7]$^+$ (44.44).
methyl tricosanoate	368 [M]$^+$ (18.88), 355 [$C_{23}H_{47}O_2$]$^+$ (0.22), 337 [$C_{23}H_{45}O$]$^+$ (2.22), 325 [$C_{21}H_{41}O_2$]$^+$ (5.55), 311 [$C_{20}H_{39}O_2$]$^+$ (2), 297 [$C_{19}H_{37}O_2$]$^+$ (0.44), 283 [$C_{18}H_{35}O_2$]$^+$ (2.22), 269 [$C_{17}H_{33}O_2$]$^+$ (3.33), 255 [$C_{16}H_{31}O_2$]$^+$ (2), 241 [$C_{15}H_{29}O_2$]$^+$ (2), 227 [$C_{14}H_{27}O_2$]$^+$ (2), 213 [$C_{13}H_{25}O_2$]$^+$ (2.22), 199 [$C_{12}H_{23}O_2$]$^+$ (4.22), 185 [$C_{11}H_{21}O_2$]$^+$ (2.66), 171 [$C_{10}H_{19}O_2$]$^+$ (2), 157 [$C_9H_{17}O_2$]$^+$ (2.22), 143 [$C_8H_{15}O_2$]$^+$ (20), 129 [$C_7H_{13}O_2$]$^+$ (8.88), 115 [$C_6H_{11}O_2$]$^+$ (3.33), 101 [$C_5H_9O_2$]$^+$ (7.11), 97 [C_7H_{13}]$^+$ (15.77), 87 [$C_4H_7O_2$]$^+$ (71.33), 74 [$C_3H_6O_2$] (100), 55 [C_4H_7]$^+$ (44.44).
methyl tetracosanoate	382 [M]$^+$ (22.22), 351 [$C_{24}H_{47}O$]$^+$ (2.22), 339 [$C_{22}H_{43}O_2$]$^+$ (6.44), 325 [$C_{21}H_{41}O_2$]$^+$ (1.11), 311 [$C_{20}H_{39}O_2$]$^+$ (1.11), 297 [$C_{19}H_{37}O_2$]$^+$ (2.22), 283 [$C_{18}H_{35}O_2$]$^+$ (3.33), 269 [$C_{17}H_{33}O_2$]$^+$ (1.11), 255 [$C_{16}H_{31}O_2$]$^+$ (2.22), 241 [$C_{15}H_{29}O_2$]$^+$ (2.22), 227 [$C_{14}H_{27}O_2$]$^+$ (2), 213 [$C_{13}H_{25}O_2$]$^+$ (0.44), 199 [$C_{12}H_{23}O_2$]$^+$ (4.44), 185 [$C_{11}H_{21}O_2$]$^+$ (3.33), 171 [$C_{10}H_{19}O_2$]$^+$ (2), 157 [$C_9H_{17}O_2$]$^+$ (2.22), 143 [$C_8H_{15}O_2$]$^+$ (22.22), 129 [$C_7H_{13}O_2$]$^+$ (8.88), 115 [$C_6H_{11}O_2$]$^+$ (2.66), 101 [$C_5H_9O_2$]$^+$ (7.77), 97 [C_7H_{13}]$^+$ (15.55), 87 [$C_4H_7O_2$]$^+$ (77.77), 74 [$C_3H_6O_2$] (100), 55 [C_4H_7]$^+$ (43.33).
methyl 14-methylhexadecanoate	284 [M]$^+$ (10), 253 [$C_{17}H_{33}O$]$^+$ (3.33), 227 [$C_{14}H_{27}O_2$]$^+$ (2.22), 213 [$C_{13}H_{25}O_2$]$^+$ (2.22), 199 [$C_{12}H_{23}O_2$]$^+$ (4.66), 185 [$C_{11}H_{21}O_2$]$^+$ (6.66), 171 [$C_{10}H_{19}O_2$]$^+$ (2.22), 157 [$C_9H_{17}O_2$]$^+$ (3.33), 143 [$C_8H_{15}O_2$]$^+$ (17.88), 129 $C_7H_{13}O_2$]$^+$ (10), 115 [$C_6H_{11}O_2$]$^+$ (4.44), 101 [$C_5H_9O_2$]$^+$ (7.77), 97 [C_7H_{13}]$^+$ (12.22), 87 [$C_4H_7O_2$]$^+$ (66.66), 74 [$C_3H_6O_2$]$^+$ (100), 55 [C_4H_7]$^+$ (40).
methyl 9-eicosenoate	324 [M]$^+$ (2.22), 292 [$C_{20}H_{36}O$]$^+$ (13.33), 281 [$C_{18}H_{33}O_2$]$^+$ (0.22), 263 [$C_{19}H_{35}$]$^+$ (1.11), 250 [$C_{18}H_{34}$]$^+$ (4.44), 237 [$C_{17}H_{33}$]$^+$ (2), 223 [$C_{16}H_{31}$]$^+$ (2.22), 208 [$C_{15}H_{28}$]$^+$ (4.44), 195 [$C_{14}H_{27}$]$^+$ (2.22), 185 [$C_{11}H_{21}O_2$]$^+$ (1.11), 179 [$C_{13}H_{23}$]$^+$ (2.22), 171 [$C_{10}H_{19}O_2$]$^+$ (1.11), 167 [$C_{12}H_{23}$]$^+$ (4.44), 157 [$C_9H_{17}O_2$]$^+$ (2), 152 [$C_{11}H_{20}$]$^+$ (4.66), 143 [$C_8H_{15}O_2$]$^+$ (3.33), 139 [$C_{10}H_{19}$]$^+$ (5.55), 129 [$C_7H_{13}O_2$]$^+$

	(4.44), 123 (11.11), 115 $[C_6H_{11}O_2]^+$ (4.44), 111 $[C_8H_{15}]^+$ (17.77), 101 $[C_5H_9O_2]^+$ (4.66), 97 $[C_7H_{13}]^+$ (40), 83 $[C_6H_{11}]^+$ (48.88), 69 $[C_5H_9]^+$ (64.44), 55 $[C_4H_7]^+$ (100).
methyl (Z,Z)7,10-methyloctadecadienoate	294 $[M]^+$ (13.33), 264 $[C_{17}H_{28}O_2]^+$ (16.66), 237 $[C_{15}H_{25}O_2]^+$ (3.33), 222 $[C_{14}H_{22}O_2]^+$ (6.88), 207 $[C_{15}H_{27}]^+$ (2.22), 193 $[C_{14}H_{25}]^+$ (2.22), 180 $[C_{13}H_{24}]^+$ (6.88), 169 $[C_{10}H_{17}O_2]^+$ (2), 164 $[C_{12}H_{20}]^+$ (8.88), 155 $[C_9H_{15}O_2]^+$ (1.11), 149 $[C_{11}H_{17}]^+$ (17.77), 143 $[C_8H_{15}O_2]^+$ (4.66), 135 $[C_{10}H_{15}]^+$ (22.22), 129 $[C_7H_{13}O_2]^+$ (6.66), 125 $[C_9H_{17}]^+$ (8.88), 115 $[C_6H_{11}O_2]^+$ (6.66), 109 $[C_8H_{13}]^+$ (31.11), 101 $[C_5H_9O_2]^+$ (6.66), 95 $[C_7H_{11}]^+$ (75.55), 81 (88.88), 67 (100), 55 $[C_4H_7]^+$ (82.66).
methyl (Z,Z)8,11-octadecadienoate	294 $[M]^+$ (6.66), 264 $[C_{17}H_{28}O_2]^+$ (6.66), 237 $[C_{15}H_{25}O_2]^+$ (1.11), 222 $[C_{14}H_{22}O_2]^+$ (2.66), 193 $[C_{14}H_{25}]^+$ (1.11), 185 $[C_{11}H_{21}O_2]^+$ (2), 180 $[C_{13}H_{24}]^+$ (3.33), 164 $[C_{12}H_{20}]^+$ (5.55), 157 $[C_9H_{17}O_2]^+$ (2.22), 149 $[C_{11}H_{17}]^+$ (10.88), 143 $[C_8H_{15}O_2]^+$ (4.22), 135 $[C_{10}H_{15}]^+$ (13.55), 129 $[C_7H_{13}O_2]^+$ (7.11), 125 $[C_9H_{17}]^+$ (11.11), 115 $[C_6H_{11}O_2]^+$ (5.55), 109 $[C_8H_{13}]^+$ (26.66), 101 $[C_5H_9O_2]^+$ (4.66), 95 $[C_7H_{11}]^+$ (58.88), 81 (79.77), 67 $[C_5H_7]^+$ (100),55 $[C_4H_7]^+$ (84.44).
methyl (Z,Z)9,12-octadecadienoate	294 $[M]^+$ (2.44), 265 $[C_{17}H_{28}O_2]^+$ (5.55), 237 $[C_{15}H_{25}O_2]^+$ (2.44), 222 $[C_{14}H_{22}O_2]^+$ (5.55), 207 $[C_{15}H_{27}]^+$ (1.11), 193 $[C_{14}H_{25}]^+$ (2), 185 $[C_{11}H_{21}O_2]$ (2.22), 180 $[C_{13}H_{24}]^+$ (6.66), 164 $[C_{12}H_{20}]^+$ (4.44), 157 $[C_9H_{17}O_2]^+$ (2.66), 149 $[C_{11}H_{17}]^+$ (11.11), 143 $[C_8H_{15}O_2]^+$ (4.44), 135 $[C_{10}H_{15}]^+$ (15.55), 129 $[C_7H_{13}O_2]^+$ (11.11), 115 $[C_6H_{11}O_2]^+$ (6.88), 109 $[C_8H_{13}]^+$ (31.11), 101 $[C_5H_9O_2]^+$ (6.88), 95 $[C_7H_{11}]^+$ (60.22), 79 (80), 67 $[C_5H_7]^+$ (98), 55 $[C_4H_7]^+$ (100).
methyl (Z,E)9,11-octadecadienoate	294 $[M]^+$ (8.88), 263 $[C_{18}H_{31}O]^+$ (4.44), 220 $[C_{16}H_{28}]^+$ (2.22), 192 $[C_{14}H_{24}]^+$ (2.22), 179 $[C_{13}H_{23}]^+$ (4.44), 164 $[C_{12}H_{20}]^+$ (6.66), 135 $[C_{10}H_{15}]^+$ (13.33), 109 $[C_8H_{13}]^+$ (26.66), 95 $[C_7H_{11}]^+$ (58.88), 81 $[C_6H_9]^+$ (84.44), 67 $[C_5H_7]^+$ (100), 55 $[C_4H_7]^+$ (64.44).
methyl (Z,Z,Z)9,12,15-octadecatrienoate	292 $[M]^+$ (3.33), 263 $[C_{17}H_{27}O_2]^+$ (5), 237 $[C_{15}H_{25}O_2]^+$ (3.33), 222 $[C_{14}H_{22}O_2]^+$ (6.66), 197 $[C_{12}H_{21}O_2]^+$ (1.66), 185 $[C_{11}H_{21}O_2]$ (5), 177 $[C_{13}H_{21}]^+$ (1.66), 163 $[C_{12}H_{19}]^+$ (3.33), 157 $[C_9H_{17}O_2]^+$ (3.33), 149 $[C_{11}H_{17}]^+$ (11.66), 143 $[C_8H_{15}O_2]^+$ (3.33), 135 $[C_{10}H_{15}]^+$ (15), 129 $[C_7H_{13}O_2]^+$ (13.33), 108 $[C_8H_{12}]^+$ (38.33), 95 $[C_7H_{11}]^+$ (61.66), 79 (100), 73 $[C_3H_5O_2]^+$ (25), 69 $[C_5H_9]^+$ (73.33), 55 $[C_4H_7]^+$ (66.66).
methyl 9-oxo nonanoate	186 $[M]^+$ (0.44), 158 $[C_9H_{18}O_2]^+$ (15.55), 155 $[C_9H_{15}O_2]^+$ (25.55), 143 $[C_8H_{15}O_2]^+$ (42.22), 129 $[C_7H_{13}O_2]^+$ (4.44), 115 $[C_6H_{11}O_2]^+$ (17.77), 111 $[C_7H_{11}O]^+$ (57.77), 101 $[C_5H_9O_2]^+$ (15.55), 98 $[C_6H_{10}O]^+$ (15.55), 87 $[C_4H_7O_2]^+$ (84.44), 73 $[C_3H_5O_2]^+$ (62.22), 59 $[C_2H_3O_2]^+$ (40), 55 $[C_3H_3O]^+$ (100).
butyl hexadecanoate	312 $[M]^+$ (8.88), 281 $[C_{18}H_{33}O_2]^+$ (0.22), 269 $[C_{17}H_{33}O_2]^+$ (2.22), 257 $[C_{16}H_{33}O_2]^+$ (28.88), 239 $[C_{16}H_{31}O]^+$ (14.44), 227 $[C_{14}H_{27}O_2]^+$ (2.44), 213 $[C_{13}H_{25}O_2]^+$ (6.66), 199 $[C_{12}H_{23}O_2]^+$ (2.22), 185 $[C_{11}H_{21}O_2]^+$ (7.77), 171 $[C_{10}H_{19}O_2]^+$ (5.55), 157 $[C_9H_{17}O_2]^+$ (4.44), 143 $[C_8H_{15}O_2]^+$ (3.33), 129 $[C_7H_{13}O_2]^+$ (24.44), 117 $[C_6H_{13}O_2]$ (13.33), 101 $[C_5H_9O_2]^+$ (12.22), 99 $[C_7H_{15}]^+$ (15.55), 83 $[C_6H_{11}]^+$ (20), 73 $[C_4H_9O]^+$ (37.77), 56 $[C_4H_8]^+$ (100).
butyl (Z,Z)9,12-octadecadienoate	336 $[M]^+$ (5.55), 279 $[C_{18}H_{31}O_2]^+$ (3.33), 263 $[C_{18}H_{31}O]^+$ (12.22), 220 $[C_{16}H_{28}]^+$ (4.44), 207 $[C_{15}H_{27}]^+$ (2.44), 199 $[C_{12}H_{23}O_2]^+$ (2.22), 193 $[C_{14}H_{25}]^+$ (2.22), 185 $[C_{11}H_{21}O_2]^+$ (2.44), 179 $[C_{13}H_{23}]^+$ (6.66), 171 $[C_{10}H_{19}O_2]^+$ (4.44), 164 $[C_{12}H_{20}]^+$ (7.77), 157 $[C_9H_{17}O_2]^+$ (2.22), 149 $[C_{11}H_{17}]^+$ (13.33), 135 $[C_{10}H_{15}]^+$ (20), 129 $[C_7H_{13}O_2]^+$ (13.33), 109 $[C_8H_{13}]^+$ (33.33), 101 $[C_5H_9O_2]^+$ (5.55), 95 $[C_7H_{11}]^+$ (70), 81 $[C_6H_9]^+$ (86.66), 67 $[C_5H_7]^+$ (100), 55 $[C_4H_7]^+$ (80).
butyl 9,12,15-octadecatrienoate	334 $[M]^+$ (4.44), 305 $[C_{20}H_{33}O_2]^+$ (1.11), 277 $[C_{18}H_{29}O_2]^+$ (2.22), 261 $[C_{18}H_{29}O]^+$ (6.66), 231 $[C_{17}H_{27}]^+$ (0.22), 191 $[C_{14}H_{23}]^+$ (3.55), 185 $[C_{11}H_{21}O_2]^+$ (2.22), 177 $[C_{13}H_{21}]^+$ (2.22), 163 $[C_{12}H_{19}]^+$ (6.66), 149 $[C_{11}H_{17}]^+$ (15.55), 135 $[C_{10}H_{15}]^+$ (20), 129 $[C_7H_{13}O_2]^+$ (7.77), 121 $[C_9H_{13}]^+$ (24.44), 108 $[C_8H_{12}]^+$ (42.22), 95 $[C_7H_{11}]^+$ (63.33), 79 $[C_6H_7]^+$ (100), 67 $[C_5H_7]^+$ (73.33), 55 $[C_4H_7]^+$ (50).
2,3-dihydroxypropyl hexadecanoate	330 $[M]^+$ (1.11), 313 $[C_{19}H_{37}O_3]^+$ (2.22), 273 $[C_{15}H_{29}O_4]^+$ (1.11), 256 $[C_{16}H_{32}O_2]$ (4.66), 245 $[C_{13}H_{25}O_4]^+$ (1.11), 230 $[C_{12}H_{22}O_4]^+$ (1.11), 213 $[C_{15}H_{33}]$ (3.33), 197 $[C_{14}H_{29}]^+$ (4.44), 185 $[C_{13}H_{29}]$ (6.66), 171 $[C_{12}H_{27}]$ (4.66), 155 $[C_{11}H_{23}]^+$ (6.66), 147 $[C_6H_{11}O_4]^+$ (6.88), 141 $[C_{10}H_{21}]^+$ (8.88), 135 $[C_5H_{11}O_4]$ (15.55), 129 $[C_9H_{21}]$ (28.88), 117 $[C_4H_5O_4]^+$ (22.22), 95 $[C_7H_{11}]^+$ (38.88), 85 $[C_6H_{13}]^+$ (47.77), 71 $[C_5H_{11}]^+$ (73.33), 57 $[C_4H_9]^+$ (100).
3-hydroxy-2-methylpropyl palmitate	328 $[M]^+$ (4.44), 313 $[C_{19}H_{37}O_3]^+$ (19.78), 300 $[C_{18}H_{36}O_3]^+$ (3.33), 285 $[C_{17}H_{33}O_3]^+$ (2.22), 269 $[C_{17}H_{33}O_2]^+$ (1.11), 256 $[C_{15}H_{28}O_3]^+$ (2.22), 213 $[C_{12}H_{21}O_3]^+$ (13.33), 201 $[C_{11}H_{21}O_3]^+$ (3.33), 185 $[C_{10}H_{17}O_3]^+$ (4.44), 156 $[C_{11}H_{24}]^+$ (17.78), 145 $[C_7H_{13}O_3]^+$ (15.56), 127 $[C_9H_{19}]^+$ (52.22), 117 $[C_5H_9O_3]^+$ (44.44), 99 $[C_7H_{15}]^+$ (34.44), 73 $[C_4H_9O]^+$ (51.11), 57 $[C_4H_9]^+$ (100).
1-monolinolenin	352 $[M]^+$ (0.44), 313 $[C_{18}H_{33}O_4]^+$ (20), 297 $[C_{17}H_{29}O_4]^+$ (1.11), 281 $[C_{16}H_{25}O_4]^+$ (2.44), 257 $[C_{14}H_{25}O_4]^+$ (2.44), 207 $[C_{15}H_{27}]^+$ (4.44), 191 $[C_{14}H_{23}]^+$ (2.22), 189 $[C_9H_{17}O_4]^+$ (2.22), 177 $[C_{13}H_{21}]^+$ (3.33), 163 $[C_{12}H_{19}]^+$ (4.67), 135 $[C_{10}H_{15}]^+$ (19.78), 117 $[C_4H_5O_4]^+$ (47.11), 109 $[C_8H_{13}]^+$ (22.22), 95 $[C_7H_{11}]^+$ (55.56), 79 $[C_6H_7]^+$ (68.89), 67 $[C_5H_7]^+$ (75.56), 55 $[C_4H_7]^+$ (100).

2,3-dihydroxypropyl octadecanoate	358 $[M]^+$ (0.44), 340 $[C_{21}H_{40}O_3]^+$ (8.89), 327 $[C_{20}H_{39}O_3]^+$ (1.11), 297 $[C_{19}H_{37}O_2]^+$ (2), 285 $[C_{16}H_{29}O_4]^+$ (15.55), 267 $[C_{18}H_{35}O]^+$ (11.11), 241 $[C_{17}H_{37}]^+$ (4.44), 227 $[C_{16}H_{35}]^+$ (2.22), 213 $[C_{15}H_{33}]^+$ (2.44), 199 $[C_{14}H_{31}]^+$ (3.33), 185 $[C_{13}H_{29}]^+$ (11.11), 171 $[C_{12}H_{27}]^+$ (4.66), 157 $[C_{11}H_{25}]^+$ (6.44), 129 $[C_9H_{21}]^+$ (37.77), 117 $[C_4H_7O_4]^+$ (25.55), 97 $[C_7H_{13}]^+$ (33.33), 85 $[C_6H_{13}]^+$ (43.33), 73 $[C_5H_{13}]^+$ (78.88), 55 $[C_4H_7]^+$ (100).
squalene	410 $[M]^+$ (1.66), 355 $[C_{26}H_{43}]^+$ (0.33), 341 $[C_{25}H_{41}]^+$ (3.33), 273 $[C_{20}H_{33}]^+$ (0.33), 207 $[C_{15}H_{27}]$ (5), 191 $[C_{14}H_{23}]^+$ (3.33), 136 $[C_{10}H_{16}]^+$ (15), 123 $[C_9H_{15}]^+$ (10), 83 $[C_6H_{11}]^+$ (60), 69 $[C_5H_9]^+$ (100), 55 $[C_4H_7]^+$ (8.33).
campesterol	400 $[M]^+$ (100), 382$[C_{28}H_{46}]^+$ (46.66), 357 $[C_{25}H_{41}O]^+$ (5), 340 (10), 315 $[C_{22}H_{35}O]^+$ (60), 289 (58.33), 273 $[C_{19}H_{29}O]^+$ (26.66), 161 (40), 145 (53.33), 123 (20), 107 (63.33), 81 (60), 71 $[C_5H_{11}]^+$ (46.66), 55 $[C_4H_7]^+$ (66.66).
β-sitosterol	414 $[M]^+$ (100), 396 (46.66), 371 $[C_{26}H_{43}O]^+$ (3.33), 354 (10), 329 $[C_{23}H_{37}O]^+$ (56.66), 315 $[C_{22}H_{35}O]^+$ (3.33), 303 $[C_{21}H_{35}O]^+$ (50), 273 $[C_{19}H_{29}O]^+$ (30), 255 (33.33), 247 $[C_{17}H_{27}O]^+$ (5), 231 (21.66), 170 $[C_{12}H_{26}]$ (16.66), 81 (56.66), 55 $[C_4H_7]^+$ (58.33).
9,19-cyclolanost-24-en-3-ol	426 $[M]^+$ (10), 411 $[C_{29}H_{47}O]^+$ (23.33), 393 (41.66), 375 $[C_{25}H_{41}O]^+$ (3.33), 365 (25), 343 $[C_{24}H_{39}O]^+$ (3.33), 339 (11.66), 315 $[C_{22}H_{35}O]^+$ (3.66), 286 (33.33), 273 $[C_{19}H_{29}O]^+$ (20), 231 (10), 207 $[C_{14}H_{23}O]^+$ (20), 109 $[C_8H_{13}]^+$ (56.66), 95 (70), 83 $[C_6H_{11}]^+$ (48.33), 69 $[C_5H_9]^+$ (100), 55 $[C_4H_7]^+$ (56.66).
3-ethylcyclohexene	110 $[M]^+$ (0.67), 95 $[C_7H_{11}]^+$ (10), 81 $[C_6H_9]^+$ (100), 67 $[C_5H_7]^+$ (33.33), 55 $[C_4H_7]^+$ (21.66).
selegiline	187 $[M]^+$ (0.67), 96 $[C_6H_{10}N]^+$ (100), 91 $[C_7H_7]^+$ (10), 77 $[C_6H_5]^+$ (1.67), 56 $[C_4H_8]^+$ (13.33).

3. EXPERIMENTAL

3.1 GC/MS: Method 1: GC/MS analysis was performed at Agriculture Research Center, National Research Center, NRC, Dokki, Cairo, Egypt, on Aglient 6890 gas chromatograph equipped with an Aglient mass spectrometric detector, with a direct capillary interface and fused silica capillary column HP-5ms (30m x 0.32 mm x 0.25 µm film thickness). Helium was used as carrier gas at approximately 1.0 ml/mim, pulsed splitess mode. The solvent delay was 3 min and the injection size was 1.0 µl. The mass spectrometric detector was operated in electron impact ionization mode with an ionizing energy of 70 eV., scanning from m/z 50 to 500. The ion source temperature was 230°C. The electron multiplier voltage (EM voltage) was maintained at 1250 V above auto tune. The instrument was manually tuned using perfluorotributyl amine (PFTBA). The GC temperature program was started at 60°C (2 min) then elevated to 280°C at a rate of 8°C/min. The detector and injector temperature were set at 300 and 280°C, respectively. Wiley and Wiley Nist mass spectral data base was used in the identification of separated peaks; **Method 2**: GC/MS analysis was performed at National Research Center (NRC), Dokki, Cairo on a varian GC interfaced to Finnigan SSQ 7000 Mass Selective Detector (MSD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J & W Scientific, Folosm, CA) cross–linked fused silica capillary column (30 m long, 0.25 mm internal diameter) coated with polydimethylsiloxane (0.5µm film thickness). The oven temperature was programmed from 50°C for 3 min., at isothermal, then heating by 7°C/ min. to 250°C and isothermally for 10 min., at 250°C. Injector temperature was 200°C and the volume injected was 0.5 µl. Transition line and ion source temperature were 250°C and 150°C respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent peak and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV.

3.2 Materials and Reagents

Solvents: petroleum ether (60-80), diethyl ether, hexane, methylene chloride, ethyl acetate, acetone, butanol and methanol were obtained from Adwic Company; **Chemical reagents for cytotoxicity activity**: The cell lines HePG-2, hepatocellular carcinoma (liver) and MCF-7 mammary gland (breast), were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt; RPMI-1640 medium, MTT, DMSO and 5-fluorouracil were obtained from Sigma co., St. Louis, USA; fetal bovine serum was obtained from GIBCO, UK; **Chromatographic materials**: preparative (PTLC) was performed on silica gel (Kieselgel 60, GF 254) of 0.25 thickness; **Materials for nanoparticle formulation**: poly (ethylene glycol)-block-Poly (propylene glycol)-block- Poly (ethylene glycol) [Pluronic F-108] and acetone were provided by Aldrich (Germany). All the other chemical reagents were of analytical grade.

3.3 Plant material

Flaxseed (Linum usitatissimum) was purchased from local market, Mansoura city, Egypt, in January 2014.

3.4 Processing of the plant material

Flaxseed was grinded to give a dried powder material (350 g), which was extracted by a soxhlet extractor using different solvents; n-hexane, methylene chloride and methanol, respectively to obtain three fractions; hexane fraction, Lu1 (118.2 g, 33.771% w/w), methylene chloride fraction, Lu2 (20 g, 5.714% w/w) and methanol fraction, Lu3 (47 g, 13.428% w/w). The methylene chloride fraction, Lu2 was defatted with cold methanol to give defatted material. The methanol fraction, Lu3 was hydrolyzed at room temperature with 1M NaOH for 12 h, then the solution was neutralized with 0.1 HCl then extracted by hexane, methylene chloride, ethyl acetate and butanol using a separatory funnel to give the subfractions hexane (Lu3a, 1.142 g), methylene chloride

(Lu3b, 1.993 g), ethyl acetate (Lu3c, 1.600 g) and butanol (Lu3d, 1.200 g), respectively.

GC/MS analysis of hexane fraction (Lu1): A sample of hexane extract (Lu 1) was analyzed using GC/MS analysis method 1 to afford 2,4-heptadienal (Rt 7.17 min., 0.17 %), dodecane (R_t 11.20 min., 0.05%), 2-decenal (R_t 12.37 min., 0.06%), 3-ethylcyclohexene (R_t 13.37 min., 0.20%), tetradecane (R_t 14.70 min., 0.04%), selegiline (R_t 15.36 min., 0.56%), pentadecanoic acid (R_t 21.44 min., 0.07%), hexadecanoic acid (R_t 23.08 min., 10.69%), 9,17-octadecadienal (R_t 23.72 min., 0.09%), heptadecanoic acid (R_t 23.93 min., 0.06%), linoleic acid (R_t 24.23 min., 0.07%), methyl (Z,Z,Z)9,12,15-octadecatrienoate (R_t 24.31 min., 0.13%), (Z,Z) 9,12-octadecadienoic acid (R_t 24.55 min., 0.35%), 9,12,15-octadecatrien-1-ol (R_t 24.69 min., 0.49%), 9,12,15-octadecatrienoic acid (R_t 25.97 min., 80.97%) , eicosane (R_t 29.90 min., 0.07%), octacosane (R_t 30.93 min., 0.08%), squalene (R_t 31.66 min., 0.09%), nonacosane (R_t 32.07 min., 0.08%), triacontane (R_t 33.37 min., 0.08%), campesterol (R_t 37.85 min., 0.16%), -sitosterol (R_t 39.90 min., 0.25%) and 9,19-cyclolanost-24-en-3-ol (R_t 41.85 min., 0.34%).

GC/MS analysis of methylene chloride fraction (Lu2): A sample of methylene chloride fraction (Lu 2) was analyzed using GC/MS analysis method 1 to afford hexadecane (R_t 31.28 min., 0.87%), heptadecane (R_t 34.05 min., 0.84%), octadecane (R_t 36.66 min., 1.15%), methyl palmitate (R_t 39.88 min., 6.19%), eicosane (R_t 41.54 min., 1.14%), 3-hydroxy-2-methylpropylpalmitate (R_t 43.24 min., 0.54%), 1-monolinolenin (R_t 43.45 min., 0.77%), methyl,9-cis,11-trans-octadecadienoate (R_t 43.79 min., 7.69%), methyl (Z,Z,Z)9,12,15-octadecatrienoate (R_t 43.95 min., 32.01%), methyl octadecanoate (R_t 44.51 min., 1.88%), butyl hexadecanoate (R_t 45.77 min., 2.88%), tetracosane (R_t 46.01 min., 0.92%), 2,3-dihydroxypropyl hexadecanoate (R_t 48.11 min., 0.65%), butyl 9,12-octadecadienoate (R_t 49.29 min., 2.45%), buty,9,12,15-octadecatrienoate (R_t 49.46 min., 16.29%), 2,3-dihydroxypropyl octadecanoate R_t 49.93 min., 0.80%), tetracosane (R_t 50.13 min., 1.19%), pentacosane (R_t 52.07 min., 0.94%), hexacosane (R_t 53.94 min., 1.16%), heptacosane (R_t 55.94 min., 1.29%), octacosane (R_t 57.48 min., 1.37%), nonacosane (R_t 59.17 min., 0.98%), triacontane (R_t 60.80 min., 0.73%) and hentriacontane (R_t 62.38 min., 0.61%).

GC/MS analysis of hexane subfraction Lu 3a: A sample of hexane subfraction (Lu 3a) was analyzed using GC/MS analysis method 2 to afford methyl-9-oxo-nonanoate (R_t 26.61 min., 0.71%), cetene (R_t 31.10 min., 0.25%), methyl tetradecanoate (R_t 34.83 min., 0.49%), 1-octadecene (R_t 36.54 min., 0.30%), methyl pentadecanoate (R_t 37.45 min., 0.40%), methyl palmitate (R_t 40.37 min., 38.64%), methyl 14-methylhexadecanoate (R_t 42.36 min., 0.28%), hexadecanoic acid (R_t 42.73 min., 0.50%), methyl (Z,Z)9,12-octadecadienoate(R_t 43.73 min., 1.75%), methyl (Z,Z)8,11-octadecadienoate (R_t 43.86 min., 0.59%), methyl (Z,Z)7,10-methyloctadecadienoate (R_t 44.37 min., 9.91%), methyl (Z,Z,Z)9,12,15-octadecatrienoate (R_t 45.00 min., 5.36%), methyl octadecanoate (R_t 45.16 min., 9.85%), (Z,Z)9,12-octadecadienoic acid (R_t 46.38 min., 4.16%), methyl-9-eicosenoate (R_t 48.38 min., 0.57%), methyl eicosanoate (R_t 48.90 min., 0.40%), methyl docosanoate (R_t 52.80 min., 0.41%), methyl tricosanoate (R_t 54.63 min., 0.22%) and methyl tetracosanoate (R_t 56.44 min., 0.62%).

GC/MS analysis of methylene chloride subfraction Lu 3b: A sample of methylene chloride subfraction (Lu 3b) was analyzed using GC/MS analysis method 2 to afford eicosane (R_t 15.10 min., 0.61%), 2-methyl-eicosane (R_t 16.76 min, 0.15%), henicosane (R_t 17.33 min, 0.41%), docosane (R_t 19.47 min, 0.49%), tricosane (R_t 21.55 min, 0.22%), tetracosane (R_t 23.56 min, 0.81%), pentacosane (Rt 25.48 min, 0.32%), hexacosane (R_t 27.34 min, 0.54%), heptacosane (R_t 29.14 min, 0.30%), octacosane (R_t 30.88 min, 0.40%), nonacosane (R_t 32.57 min, 0.24%), triacontane (R_t 34.19 min, 0.22%), hentriacontane (R_t 35.77 min, 0.15%) and dotriacontane (R_t 37.33 min, 0.10%).

Chromatographic separation trials of subfractions (hexane, methylene chloride and ethyl acetate) didn't give pure compounds.

The subfraction (Lu 3d, 1.200g) was subjected to silica gel CC using ethyl acetate/methanol as an eluent with gradient increasing polarity. The eluted fractions were monitored and collected based on their TLC patterns. The fraction eluted by ethyl acetate /methanol 7:3 (0.120 g) was reseparated on a sephadex LH-20 CC (methylene chloride/methanol, 1:9) to give 8–5' neolignan (dehydrodiconiferyl alcohol-4-β-D-glucoside **1** , 55 mg).

3.5 Preparation of butanol subfracrion Lu 3d -loaded pluronic nano-micelles

Lu 3d-loaded pluronic nano-micelles were prepared via nanoprecipitation method. The used polymer: Lu 3d ratio was 2:1. In brief, 124 mg of pluronic and 62 mg of the the Lu 3d were dissolved in 15 ml acetone (95%) to prepare the organic phase. After complete dissolution of the organic phase, it was added dropwise with sonication into 30 ml of distilled water. The final suspension was left to allow the evaporation of acetone and a water portion to form a concentrated nanosuspension. Finally, the obtained nanosuspension was lyophilized in a Labconco freeze dryer to obtain dried nanoparticles powder. Plain pluronic nanoparticles were prepared by the same method as control. The entrapment efficiency (EE%) of the loaded Lu 3d was determined by centrifugation of a certain volume of the nanoparticles suspension at a speed of 24,000 rpm with cooling. Then, the amounts of the unloaded Lu 3d remaining free in the supernatant were determined using UV-Vis spectrophotometry at the optimum wavelengths. Afterwards, the amount of the Lu 3d loaded into the nanoparticles was obtained by knowing the initial added amount of the Lu 3d. The mean value from three replicates ± SD was obtained. The entrapment efficiency (*EE %*) of the Lu 3d was calculated according to the following relationship:

$$EE\ \% = \left(\frac{mr}{mi}\right) \times 100$$

Where m_i and m_r are the amounts (mg) of the bioactive compound initially loaded and remained in the nanoparticles, respectively.

3.6 Determination of particle size and zeta potential

The size and the zeta potential of the prepared plain and Lu 3d-loaded pluronic nanoparticles were estimated at λ_{max} of 480 nm using dynamic light scattering, DLS (Malvern nanosizer, Malvern Instruments Ltd., Worcestershire, UK) with a refractive index of 1.363 for the solvent.

3.7 Surface morphology

The morphology of the developed pluronic nanoparticles was examined by the transmission electron microscopy (TEM, JEOL, JEM-1230, Japan Ltd.) at an accelerating voltage of 200 kV. Samples were prepared for the imaging via drying the nanoparticles on a copper grid that is coated with a thin layer of carbon.

3.8 *In-vitro* enzymatic degradation study

An *in-vitro* degradation study of the developed pluronic nanoparticles was carried out in presence of lysozyme (1.5

mg/ml PBS, pH 7.4). A certain weight (W_0) of the nanoparticles (10-15 mg) was transferred to microcentrifuge tube and incubated with 0.8 ml of lysozyme solution at 37°C in a shaking incubator at 120 rpm (VWR® incubating orbital shaker, VWR International, Brisbane, CA, USA) for 60 min. The samples were then centrifuged for 2 min at speed of 14000 rpm and the final weight of the nanoparticles (W_t) was determined after discarding the supernatant. Afterward, a fresh lysozyme solution (0.8 ml) was added to the nanoparticles. At certain intervals, the steps of centrifugation and weighing were repeated and the final weights (W_t) of nanoparticles at these intervals were determined. The percent weight remaining ($W_r\%$) of the samples due to enzymatic degradation were calculated according to the following relationship:

$$W_r\ (\%) = 100 - \left(\frac{\left(W_0 - W_t\right)}{W_0} \times 100 \right)$$

Where W_0 and W_t are the initial weight of sample, and after incubation with lysozyme for a given time, *t*, respectively.

3.9 *In-vitro* release studies

The *in-vitro* release pattern of the loaded Lu 3d from the pluronic nanoparticles was determined by transferring certain amounts (20-25 mg) of the Lu 3d-loaded nanoparticles to scintillation vials containing 10 ml of PBS, pH 7.4. The samples were maintained at 37°C in a shaking incubator at 120 rpm (VWR® incubating orbital shaker, VWR International, Brisbane, CA, USA). At predetermined intervals, 1 ml aliquots were withdrawn and diluted with an equal volume of ethanol and then analyzed at the optimum λ_{max} with the aid of UV-Vis spectrophotometer. The withdrawn aliquots were replaced with equal volumes of fresh PBS, 7.4 buffer, to keep the volume of release medium constant. The amounts of bioactive compounds released (µg) from the pluronic nanoparticles were calculated with the aid of a standard curve in PBS, pH 7.4/ethanol (1:1). The obtained results were calculated in terms of cumulative release (%, w/w) relative to the already entrapped weight of the Lu 3d in the nanoparticles. The collected data represents mean ± SD from three independent release experiments.

3.10 Statistical analysis

The obtained data was analyzed and expressed as mean ± SD. The effect of various parameters on the characteristics of the prepared pluronic nanoparticles were statistically analyzed by one-way ANOVA using Excel (Microsoft Office 2007). Differences were considered significant at the level of $p < 0.05$.

3.11 Antimicrobial activity assessment

Chemical compounds were individually tested against *Staphylococcus aureus*, as a Gram positive bacterium, *Escherichia coli,* as a Gram negative bacterium and *Candida albicans,* as a fungi. Each of the compounds was dissolved in DMSO and solutions of the concentration 1 mg /ml were prepared. Paper discs of Whatman filter paper with standard size (5 mm) were cut and sterilized in an autoclave. The paper discs were soaked in the desired concentration of the complex solution and places aseptically in the Petri-dishes containing nutrient agar media (agar 20g + beef extract 3 g + peptone 5 g) seeded with *Staphylococcus aureus*, *E. coli* and *Candida albicans*. The Petri-dishes were incubated at 36°C and the inhibition zones were recorded after 24h of incubation. Each treatment was replicated three times. The antibacterial activity of a common standard antibiotic, Ampicillin, and antifungal, Colitrimazole, was also recorded using the same procedure as above at the same concentration and solvents. The % activity index for the complex was calculated by the formula as under:

$$\%\ \text{Actiity Index} = \frac{\text{Zone of inhibition by test extract (diametre)}}{\text{Zone of inhibition by standard (diametre)}} \text{X } 100$$

3.12 Antioxidant activity assessment

For each of the investigated compounds (2 mL) of ABTS solution (60 µM) was added to 3 mL MnO_2 solution (25 mg/mL), all prepared in (5 mL) aqueous phosphate buffer solution (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered and the absorbance of the resulting green blue solution (ABTS radical solution) at 734 nm was adjusted to approx. ca. 0.5. Then, 50 µl of (2 mM) solution of the tested compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in color intensity was expressed as inhibition percentage. L–ascorbic acid was used as standard antioxidant (Positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of tested compounds. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) only.

3.13 Cytotoxicity assay

The cell lines HePG-2 and MCF-7, were used to determine the inhibitory effects of extracts on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. HepG2 was cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100 µg/ml streptomycin at 37°C in a 5% CO_2 incubator. The cell line was seeded in a 96-well plate at a density of 1.0×10^4 cells/well, at 37°C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800). The relative cell viability in percentage was calculated as:

Relative cell viability = ($Absorbance_{570}$ of treated samples/ $Absorbance_{570}$ of untreated sample) X 100.

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