Some Bioactive Fatty Derivatives from *L. Pterodonta*

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Abstract: The aerial part of *Laggera pterodonta* (DC.) Sch. Bip. (Asteraceae) was extracted successively with hexane and ethyl acetate, and subjected to chemical and microbiological investigations. Chromatographic separation of the extracts led to the isolation of five fatty derivatives identified as 2-triacontoxyethyleicosanoate, triacontyl methyl ether, ethane-1,2-dieicosanoate, eicosanoic acid, ethane-1,2-di-eicosenoate based on their spectral and physicochemical characteristics. The compounds were screened for antimicrobial activities against selected microorganisms, which include *Staphylococcus aureus* (NCTC 6571), *Bacillus subtilis* (NCTC 8236), *Klebsiella pneumonia* (ATCC 10031), *Staphylococcus aureus* (ATCC 13704), and clinical isolates of *Staphylococcus aureus, Streptococcus faecalis*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella ozaenae* and *Shigella dysenteriae*. The compounds were found to exhibit selective activities against some of the organisms with a minimum inhibitory concentration (MIC) of between 25 and 100 µg/ml and a minimum bactericidal concentration of (MBC) of 100 and 200 µg/ml. The study justified the use of the plant as antibiotic in ethnomedicinal applications, and underscores the important role fatty compounds play in cellular integrity.
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1. Introduction

The plant *Laggera pterodonta* (Figure 1) remains a very interesting wonder plant in ethnomedicine both in Asia and Africa. Belonging to the genus *Laggera* and the subfamily of Tubuliflorae of the Asteraceae (Compositae) family, it continues to generate research interest amongst natural product scientists for its numerous ethno-therapeutic application, reservoir of chemical compounds and multifaceted bio-activities. Previous taxonomic classification placed the genus under *Blumea* and infact are closely related to members of that genus. But recent classification put it as subgenera in *Inuleae* (*Inula*) genus (Noyes, 2007). *Laggera pterodonta* (DC.) Sch. Bip. and *Laggera alata* (or *L. aurita*) (D. Don) Sch. Bip. are the only well-known species of the genus in Nigeria (Egharevba et al., 2009). In China the plant is used as anti-inflammatory agent for treatment of hepatitis, arthritis, bronchitis and nephritis (Shi *et al.*, 2007; Wu *et al.*, 2007; Wu *et al.*, 2006a). In Nigeria and Cameroon, it has been reported for use in pediatric malaria, pneumonia (Adesomoju, 1999), cough and wounds (Okhale *et al.*, 2010; Wudil, 2009), and preservation of seeds against insects' attack (Ngamo *et al*., 2007; Njan-Nloga *et al*., 2007).

Extensive studies of *Laggera pterodonta* have led to the identification of many compounds, including monoterpenes, sesquiterpenes, triterpenes, cyclitols, and flavonoids in the plant (Egharevba *et al.*, 2012a, 2009; Haile, 2007). Asian scientists particularly from China, India and Pakistan, have reported various pharmacological activities on the crude extracts of the plant, and isolated a number of bioactive compounds. Some of the works reported by the early researchers include hepatoprotective activity of the total flavonoid, antiviral properties, anti-inflammatory properties, antinociceptive properties, acute toxicity and insecticidal properties (Li *et al*., 2007; Ngamo *et al.*, 2007; Shi *et al*., 2007; Wu *et al.*, 2007; Wu *et al.*, 2006b,c; Zhao *et al.*, 1997; Li and Ding, 1996). A number of eudesmane sesquiterpenoids have also been reported by these early researchers (Yang *et al*., 2007; Wu *et al*., 2006a; Fraga, 2004; Xiao *et al*., 2003; Zhao *et al*., 1997; Li and Ding, 1996). The plant has also been found to be rich in essential oil, which gives it the characteristic aroma (Egharevba *et al.,* 2012b).

Like most other medicinal plants, *L. pterodonta* have molecules of fixed fats and oils, which are esters of higher fatty acids and polyhydric alcohol, mainly glycerol. Glycerol esters are usually called glycerides or triglycerides resulting from the combination of one unit of glycerol with three units of fatty acids. They are usually associated with esters of long-chain fatty alcohols like C_{30} (acontane group), free fatty alcohol and free fatty acids.

Figure 1: ***Laggera pterodonta***

The more general term for the fixed oils, 'lipids', embraces a variety of chemical substances which include mono- and diglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty alcohols, fatty acids, fat-soluble vitamins and waxes (Evans, 2002; Sebedio *et al.*, 1996). Classification into these subclasses depends on the nature of building blocks and final ester formed. While fats consist almost entirely of esters, waxes on the other hand, often contain appreciable quantity of free acids, hydrocarbons, free alcohols and sterols, in addition to esters and ethers of the cetyl palmitate type (Evans, 2002).

The roles of fats and oils in biological systems have been well reported in literature, and ranges from cellular protection to temperature regulation (Evans, 2002; Sebedio *et al.*, 1996). Olive oil has been suggested to have protective action against colonic carcinogenesis by virtue of its action on prostaglandins in rat (Evans, 2002). Fixed and volatile oils are mostly used traditionally as emollient, purgatives, anti-feedants, insecticides, and fragrance (Evans, 2002). The antimicrobial activities of fats and fatty acids have been reported by various workers (Soluchana and Bakiyalakshmi, 2011; Wang *et al.*, 2009; Agoramoorthy *et al.,* 2007; Lieberman *et al.,* 2006; Carballeira *et al*., 1997; Petschow *et al*., 1996; Plosker and Brogden, 1996). Shimada and co-workers have reported that glycerides were more active than the free fatty acids (Shimada *et al*., 1997). Although previous involvement of fats in pharmaceutics have been focused on use as emollient, as delivery vehicle of active principles, and excipients, recent discoveries has shown that some of these molecules are themselves bioactive and could function pharmacologically to mitigate certain disease conditions (Shimada *et al*., 1997). This is especially so in crude systems such as in herbal recipes and active crude extracts with many losing their crude activity when defatted due to loss of synergistic action (Egharevba *et al*., 2010; Sofowora, 2008; Evans, 2002). Fats are believed to function by interfering with the cell metabolism, oxidative degenerative process and acting as antioxidants, or through the membranes transport system, affecting membrane fluidity, membrane enzymes and deranging the lipoproteins and glycolipids transport of the target cell (Fukuda *et al*., 2013; Youdim *et al.*, 2000). This interference, which may affect the synthesis of important components of the cell, certain membrane transport system for other molecules or cause a disruption of the structural and functional integrity of the membrane, lead to cellular leakage and eventual cell death (Youdim *et al.*, 2000).

L. pterodonta extracts have been proposed to exhibit its action by synergetic actions of certain molecules believed to be in the plant (Egharevba *et al.,* 2010). Despite the numerous works done on the plant, none has reported any bioactive fatty molecules. This is probably as a result of the fact that the research output of substances used as drugs or drug candidates have been dominated by non-fatty substances mainly, alkaloids, flavonoids, anthocyanidines, saponins, cardiac-glycosides, carbohydrates, proteins and peptides, which as always, have tilted drug discovery research towards non-fatty substances in the plant. Hence, this study aims to isolate and identify some of the bioactive fatty substances in the plant extract.

2. Materials and Methods

Materials

The aerial part (stem and leaf) of *Laggera pterodonta* (Dc) Sch. Bip. plant was collected from Chaza village in Niger State in 2009.

All reagents used in this study were of Analar grade, and unless otherwise stated, were sourced from Zayo-Sigma Abuja, Nigeria. However, reagents used in highly sensitive analytical equipment like NMR were as specified by the equipment's manufacturer. The media used in the bioassays were of Oxoids Limited Basingstoke, Hampshire, England.

The organisms used in the study were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria, Kaduna State, Nigeria.

Melting points (mp) were taken on Barnstead Electrothermal BI 9100 and were uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were run on a Bruker AV 300 (400MHz) and DRX 500 (500MHz) spectrometers using $CDCl_3$ as solvents, and TMS as internal standard, at the University of Strathclyde Glasgow, and values are in δ (ppm). The coupling constants (J) were calculated in Hz. The IR spectra were carried out at the National Research Institute for Chemical Technology (NARICT) Zaria, on a Shimadzu Fourier Transformed Infra-Red spectrometer (FTIR) model 8400S, and values are in wave number (cm^{-1}). The GCMS were done at the same Institute on a Shimadzu GCMS-QP2010 Plus (Japan). The UV spectra were run on a Shimadzu UV-160A at the National Institute for

Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria, and the wavelengths for maximum absorption (λ_{max}) are recorded in nanometers (nm). Column chromatographic separations were performed on ChemGlass and Kontes glass bulb-columns.

Methods

The plant was identified by the taxonomist at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja. A voucher specimen with the number NIPRD/H/6403 was deposited at the Institute's herbarium for future reference. The collected plant was checked for foreign matters, which were removed. The plant was thereafter air-dried in a shade for two weeks. The dried plant was pulverised in a mortar and pestle and kept in an air-tight cellophane bag and preserved in the dark until required. The pulverised plant was subjected to successive maceration using hexane and ethyl acetate over 48hr each. The extracts were concentrated to dryness under reduced pressure and kept in a cool and dark place until required.

Column chromatographic separation and isolation of Compounds from the extracts.

The hexane extract was adsorbed on silica gel and chromatographed on a glass-column of silica gel. A gradient-mixture of n-hexane, ethyl acetate and methanol were employed as the elution solvents. Eluates were collected in volumes of 150-200 ml and a total of 50 fractions were collected. The fractions were labelled LPH_1 to LPH_{50}. Fractions LPH_{4-6} (100% hexane) yielded HOE 20.

The ethyl acetate extract was also adsorbed on silica gel and chromatographed on a glass-column using a gradient-mixture of n-hexane, ethyl acetate and methanol as elution solvents. A total of 36 fractions were collected in volumes of 150-200 ml. The fractions were labelled LPE_1 to LPE_{36}. LPE_{5-6} (10% ethyl acetate in hexane) yielded compound HOE 26. LPE_7 (15-20% ethyl acetate in hexane) yielded HOE 25B, while LPE_{8-13} (20-30% ethyl acetate in hexane) yielded HOE 3D and HOE 3E after re-fractionation and recrystallization in methanol.

Bioassay of extractives

The microbiological assays were done according to the methods outlined in Egharevba *et al.*, 2009.

3. Results and Discussion

The results of physicochemical characteristics and spectral analysis of the isolated compounds are shown in Tables 1-3, while the results of bioassay of are in Tables 4 and 5.

Table 1: Physical description, melting points, yields, TLCs and solubility of isolated compounds

Cpds HOE	Description	M.p. (°C)	*Yields (mg) (%)	TLC (R_f/solvent system/Plate)	Solubility (Solvents)
3D	White flakes	76-77.0	33.00 (0.07%)	0.21/Hex:EtoAc=10:1/NP	Chl, Hex.
3E	White plates	75.5-76.0	30.00 (0.06%)	0.39 /Hex: EtoAc= 10:3/NP	Chl, Hex.
20	White flakes	67.5-68.0	33.80 (0.17%)	0.76 /Hex: EtoAc= 10:0.5/NP	Chl, Hex.
25B	White crystals	76-76.5	63.30 (0.13%)	0.39 /Hex: EtoAc= 10:3/NP	Met, EtoAc, Chl.
26	White flakes	58.5-59.0	48.20 (0.10%)	0.76 /Hex: EtoAc= 10:0.5/NP	Chl, Hex.

Key: Cpd = Compounds; M.p = Melting points; NP = Normal-Phase plates; RP = Reverse-Phase plate; Hex = n-Hexane; EtoAc = Ethyl acetate; Met = Methanol; Chl = Chlorofom; Dichl = Dichloromethane; *Yield = % yields was based on the weight of extracts and purified compounds.

Table 2: ^{1}H NMR data of compounds HOE 3E, HOE 25B and HOE 3D

Carbon No.	HOE 3E	HOE 25B	HOE 3D
1	3.66 t	-	-
2	1.58 m	2.37 t, J=9.3, 9.45	2.36 m
3	,,	1.62-1.70m	1.63 m
4	,,	1.27 s	,,
5	,,	,,	1.28 s
6-19	1.27 s	,,	,,
20	,,	0.91 t J=8.3, 8.8	,,
21-29	,,	-	,,
30	0.90 t, J=6.95	-	0.90 t
1' and 3'	3.51 s	-	4.07 t
1"			3.67m

Table 3: ^{1}H and ^{13}C NMR data of compounds HOE 20 and HOE 26

Carbon	HOE 20		HOE 26	
	^{13}C	^{1}H	^{13}C	^{1}H
1		-	174.2	-
2	34.4	2.30 t	34.4	2.30 t
3	25.94	1.63 t	25.17	1.63 td
4	29.56	,,	,,	
5	29.57	1.28 s		1.28 s
6	29.60	,,		,,
7	29.65	,,		,,
8	29.16			
9			122.1	5.15-5.21 t
10				4.49-4.64
11-15	29.47	,,	25.9	1.28
16	29.65	,,		,,
17	25.04	,,		,,
18	31.92	,,	31.92	,,
19	22.68	,,	22.68	,,
20	14.13	0.90 t	14.18	0.89 m
1’	64.3	4.07	64.39	4.07
2’	64.3	4.07	64.39	4.07

Table 4: Results of antimicrobial screening of isolated compounds – minimum inhibitory concentration (µg/ml)

Organisms	Compounds				
	HOE 3D	HOE 3E	HOE 20	HOE 25B	HOE 26
Sa^{1}	-	-	-	25	50
Bs^{1}	50	50	-	-	-
Kp^{1}	100	50	50	50	50
Sa^{2}	-	-	-	50	-
Sa	100	-	50	50	-
Sf	-	-	-	50	-
Bs	50	-	50	-	-
Bc	100	50	50	50	50
Ec	-	-	-	100	-
Sd	-	-	-	50	-
Kp	100	50	-	50	50
Ko	100	50	50	50	50

Key: Sa^{1}=*Staphylococcus aureus* (NCTC 6571); Bs^{1} = *Bacillus subtilis* (NCTC 8236); Kp^{1} = *Klebsiella pneumoniae* (ATCC 10031); Sa^{2} = *Staphylococcus aureus* (ATCC 13704); Sa = *Staphylococcus aureus* (clinical isolates); Sf = *Streptococcus faecalis* (clinical isolates); Bs = *Bacillus subtilis* (clinical isolates); Bc = *Bacillus cereus* (clinical isolates); Ec = *Escherichia coli* (clinical isolates); Kp = *Klebsiella pneumoniae* (clinical isolates); Ko = *Klebsiella ozaenae* (clinical isolates); Sd = *Shigella dysenteriae* (clinical isolates).

HOE 3D showed antimicrobial activities against *B. subtilis* (NCTC 8230), *K. pneumoniae* (ATCC 10031) and clinical isolates of *S. aureus, B. subtilis, B. cereus, K. pneumoniae* and *K. ozaenae* (Tables 4 and 5). The compound was characterized as white papery substance; melting point 76-77°C; R_f 0.21 (Table 1); The ^{1}H-NMR showed peaks at 2.31-2.39 (4H, m, H-2), 1.62-1.65 (12H, m, H-3 , H-4), 1.27 (114H, s, H-5– H-19), 0.90 (9H, t, J = 5Hz, H-30), 4.07 (4H, t, J=7.35, 8.5Hz, H-1’, H-3’), 3.67 (2H, m, H-2’, H-1’’) (Table 11 and Fig. 9c). The proton peaks at 4.07, 3.66 and 2.31-2.39 were suggestive of an ester and an ether linkage with an ethane-1,2-diol (www.lipidlibrary.co.uk). There was also no olefinic proton. The protons count was suggestive of medium to long chain fatty acids. The compound was thus assigned 2-triacontoxyethyleicosanoate (Figure 2). The compound showed UV absorption at 246, IR absorption peaks at 2933 (C-H stretch), 1724 (C=O), 1459, 1382 (C-H bend), 1184 (C-O), 732 and EIMS *m/z* (% relative intensity) 41(45), 43(90), 57(100), 83(80), 97(80), 111(45), 125(20), 139(8), 153(5), 167(3), 181(2), 195(3), 209(2), 223(2), 237(2), 251(2), 265(2), 278(2), 292(2), 306(2), 320(2), 392(2), 420(2).

Table 5: Results of bactericidal screening of isolated compounds - minimum bactericidal concentration (µg/ml)

Organisms	Compounds				
	HOE 3D	HOE 3E	HOE 20	HOE 25B	HOE 26
Sa[1]	-	-	-	100	200
Bs[1]	200	200	-	-	-
Kp[1]	200	200	100	200	100
Sa[2]	-	-	-	200	-
Sa	200	-	200	200	-
Sf	-	-	-	200	-
Bs	200	-	200	-	-
Bc	200	200	200	200	200
Ec	-	-	-	200	-
Sd	-	-	-	200	-
Kp	200	200	-	200	200
Ko	200	200	200	200	200

HOE 3E inhibited the growth of *B. subtilis* (NCTC 8230), *K. pneumoniae* (ATCC 10031), and clinical isolates of *B. cereus, K. pneumoniae* and *K. ozaenae* with an MIC of 50 µg/ml and MBC of 200 µg/ml (Tables 4 and 5). The compound was characterized as a white flake, melting point 75.5-76°C; R_f = 0.39 (Table 1); The ^{1}H-NMR showed peaks at 3.66 (2H, m, H-1), 1.57-1.59 (8H, m, H-2 - H-5), 1.27(48H, s, H-6 – H-29), 0.90 (3H, t, J = 6.95, H-30), 3.51 (3H, s, H-1') (Table 2). The methyl singlet in the ^{1}H NMR spectrum at 3.51 suggests the presence of a methoxyl group while the triplet at 3.66 was suggestive of oxy-methylene. These two proton peaks suggests the compound to be ether. The other proton peaks at 1.57-1.59 and 1.27, and the MS peaks separation by *m/z* 14 (for CH_2), suggest the ether to be a long chain of many methylene groups. The proton count suggested methyl ether of triacontanol. The compound was thus assigned as triacontyl methyl ether (Figure 2). IR absorption peaks at 3020, 2924 (C-H stretch), 1363 (C-H bend), 1217 (C-O), 1041, 760, 668; UV absorption at 240-248; EIMS *m/z* (% relative intensity) 41(45), 43(90), 57(100), 83(85), 97(80), 111(45), 125(20), 139(8), 153(5), 167(3), 181(2), 195(2), 209(2), 223(2), 237(2), 251(2), 265(2), 278(2), 292(2), 306(2), 364(2), 392(2). The IR, UV and MS data further confirmed the compound to be long-chain fatty ether.

HOE 20 inhibited the growth of *K. pneumoniae* (ATCC 10031) and clinical isolates of *S. aureus, B. subtilis, B. cereus* and *K. ozaenae* (Tables 4 and 5). The compound was characterized as white flakes, melting point 67.5-68°C and R_f 0.76 (Table 1). It absorbs under UV at 244 nm. The IR spectrum showed peaks at 3021, 2933 (C-H stretch), 1714 (C=O), 1594, 1427 (C-H bend), 1217 (C-O), 1032, 760, 651, 467. The ^{1}H-NMR spectrum (Table 3) showed peaks 2.29-2.32, 1.61-1.65, 1.27, 0.90 and 4.07, while the ^{13}C-NMR spectrum (Table 3) showed peaks at 174.1 34.43, 31.92, 29.65, 29.60, 29.57, 29.56, 29.16-29-47, 25.94, 25.04, 22.68 and 14.13. There were no olefinic proton signals in the ^{1}H NMR spectrum, which suggests the compound may be a saturated compound. The proton peaks at 4.07 and 2.29-2.32 were suggestive of a glycol (ethane-1,2-diol) ester. Based the above spectral data, proton count and comparison with HOE 3D, the compound was assigned di-eicosanyl glycol or ethane-1,2-dieicosanoate (Figure 2).

HOE 25B exhibited good activity against *S. aureus* (NCTC 6571) and the clinical isolates with an MIC of 25 and 50µg/ml respectively. It also inhibited the growth of *K. pneumoniae, K. ozaenae, B. subtilis, B. cereus, E. coli, S. faecalis* and *S. dysentariae* (Tables 4 and 5). The ^{1}H NMR of HOE 25B showed peaks at 2.37 (2H, t, J=9.3, 9.45Hz), 1.62-1.70 (2H, m), 1.27(34H, s,) and 0.91 (3H, t, J = 8.3, 8.8Hz), which is characteristic of fatty acids (Table 2). However there was no olefinic proton. The compound was isolated as white flakes; melting point 76-76.5°C; R_f 0.39; and IR absorption at 3400 (OH), 2920 (C-H stretch), 1712, 1363 (C-H bend), 1218 (C-O), 1038, 661. The compound also exhibit UV absorption at 247-292 (Table 8). The EIMS spectrum gave peaks at *m/z* (% relative intensity) 41(65), 43(100), 55(65), 57(95), 73(55), 87(45), 97(30), 281(2), 340(40) (Fig. 8b). The EIMS pattern exhibited by the compound is characteristic of fatty acids. The base peak *m/z* 43 was probably due to cleavage at C-17 to yield propyl radical C_3H_7, while *m/z* 57 was due to cleavage at the α-carbon corresponding to C_2HO_2, which is characteristic of fatty acids. The above spectral data and melting point suggests compound HOE 25B to be arachidic acid or eicosanoic acid (Figure 2) (Isbell and Muud, 1998; www.lipidlibrary.co.uk). The compound was assigned eicosanoic acid with the molecular formular $C_{20}H_{40}O_2$ and molecular weight of 312. The IR absorption at 3400 due to the acid hydroxyl and the carbonyl absorption at 1712 supported the structure proposed for HOE 25B. The antimicrobial activities of long and medium chain fatty acids have been reported by workers especially in the dairy industry. Fatty acids

have been reported to exhibit anti-inflammatory activities, and growth inhibitory activities against organisms in the rhumen of the cattle, causing indigestion (Soluchana and Bakiyalakshmi, 2011; Gautam and Jacbak, 2009). Eicosanoic acid has been reported to exhibit both anti-imflammatory and antifungal activities, and also play important role as precursor in the synthesis of eicosanoids that serve as intracellular and extracellular signals (Soluchana and Bakiyalakshmi, 2011; Youdim *et al*., 2000).

HOE 26 inhibited the growth of *S. aureus* (NCTC 6571), *K. pneumoniae* (ATCC 10031) and clinical isolates of *S. aureus, B. cereus, K. pnuemoniae* and *K. ozaenae* (Tables 4 and 5). The compound was characterized as white flakes, melting point 58.5-59.0°C; R_f 0.76 (Table 1). It absorbs under UV at 248 nm. The IR spectrum showed absorption band at 2924 (C-H stretch), 1734 (C=O), 1459, 1371 (C-H bend), 1182 (C-O), 1028, 730. The ^{1}H-NMR spectrum showed peaks at 0.90, 1.28, 1.61-1.65, 2.29-2.32, 4.07, 4.49-4.64 and, 5.15-5.21, while the ^{13}C-NMR spectrum showed chemical shifts at 14.13, 22.68, 25.05, 25.94, 29.16-29.47, 29.57, 29.60, 31.92, 34.43, 122.19 and 174 (Table 3). The proton and carbon NMR spectra showed the presence of olefinic protons and carbons at 5.15-5.21, 4.49-4.64 and 122.19, 107.11, respectively, which suggest the presence of unsaturated double bond. The carbonyl carbon showed signal at 174.21, suggesting the compound to be an ester. The spectral data compares well with those reported for unsaturated fatty esters (Isbell and Muud, 1998). The compound was thus assigned ethane-1,2-di-eicosenoate (Figure 2) on the basis of proton count.

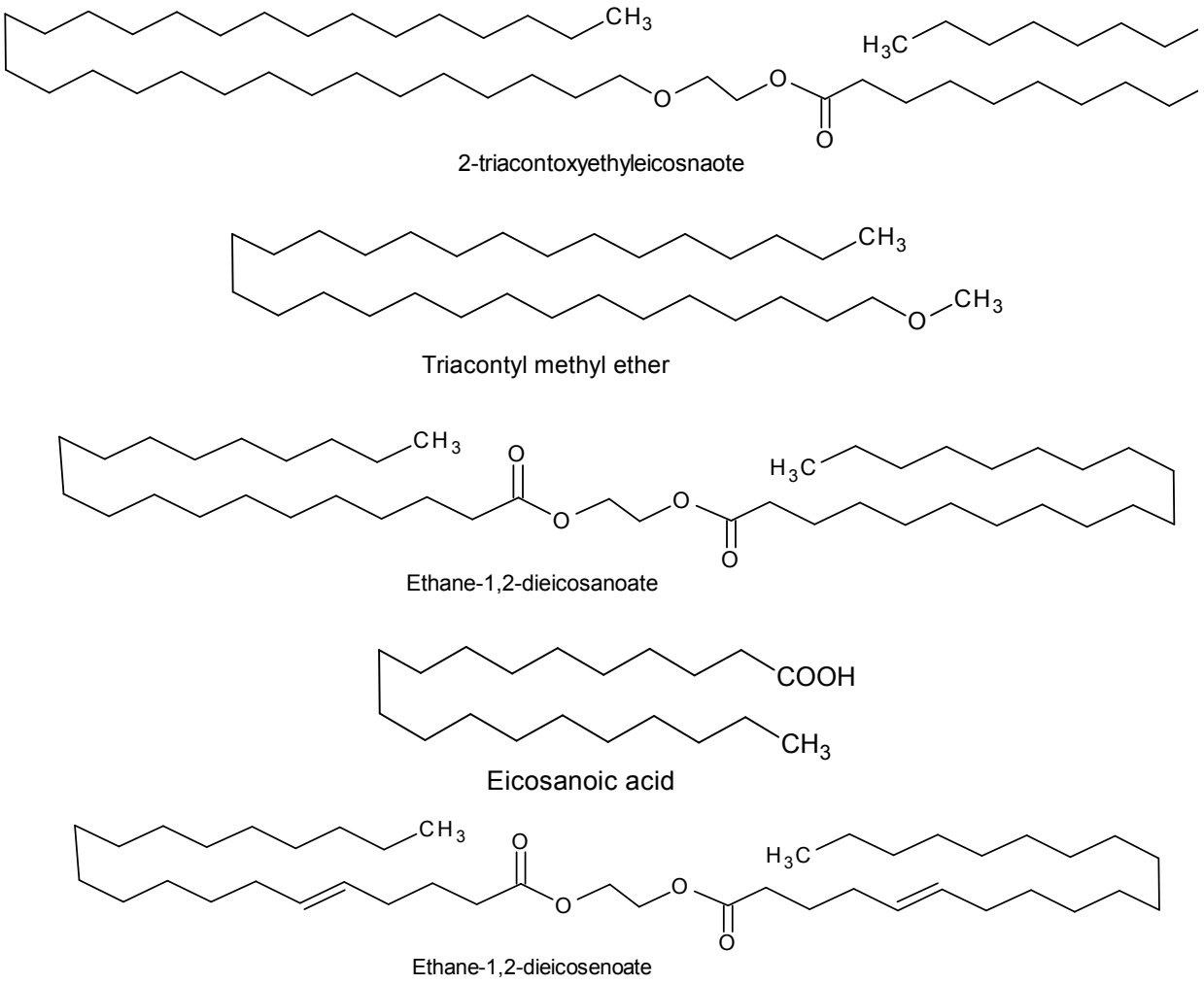

Figure 2: Structures of isolated fatty compounds

4. Conclusion

Laggera pterodonta remains a wonder plant to many researchers in the field of medicinal chemistry due to its richly endowed reservoir of pharmacologically active compounds. Over 50 compounds have been reported from the plant and scientist keep discovering new one by the day. The plant has shown to be a reservoir of terpenoids, flavonoids and glycosides (Wu *et al.*, 2006c). This study has also revealed that the plant has active lipids which may be acting in synergy with other compounds to exert pharmacological activity (Youdim *et al.*, 2000). This is the first time 2-triacontoxyethyleicosanoate, triacontyl methyl ether, ethane-1,2-dieicosanoate, eicosanoic acid, ethane-1,2-di-eicosenoate which are antibacterial will be reported from the plant, and the first time 2-triacontoxyethyleicosanoate, triacontyl methyl ether, ethane-1,2-dieicosanoate, ethane-1,2-di-eicosenoate will be reported as antimicrobials.

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