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## Some volatile constituents of the unripe fruits of *Nauclea latifolia* (Family: Rubiaceae)

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### Abstract

Fractionation, separation and purification of the ethyl acetate extract of the unripe fruits of *N. latifolia* (family Rubiaceae) using series of flash chromatography led to the isolation of a fatty acid, (palmitic acid) and two fatty acid esters (ethyl palmitate and ethenyl pentadecanoate). Characterization and structural elucidation of these compounds was achieved using UV, IR, proton-NMR, carbon 13-NMR, GC-MS, melting points and literature search.

**Keywords:** *N. latifolia*, Unripe Fruits, Palmitic acid, Ethyl palmitate, Ethenyl pentadecanoate.

### 1. Introduction

The use of natural products, such as plants, as an important source of phytochemicals for the treatment of various medical conditions is historical. They form the backbone of traditional medicine and quite a lot have been proved to possess good biological activity. Phytochemical investigation into such plants has most often led to the isolation, elucidation and characterization of lead biologically active compounds of therapeutic value [1]. Therefore, there arises the need and search for more biologically active compounds amongst these plants, especially with the emergence and spread of antibiotic resistant organisms coupled with high cost and scarcity of synthetic drugs. *Nauclea latifolia*, a natural product of the family 'Rubiaceae' is a shrub or small tree, native to tropical Africa. It is made of fruits, which has several brownish seeds embedded within it and surrounded by a pink, edible, sweet-sour pulp. The fruits are usually red and fleshy when ripe, resembling hard strawberry and yellow when unripe [2]. Traditionally, the plant has been reported useful as an antimalarial [3, 4], a purgative [5], antihypertensive [6], antibacterial [7, 8, 9, 10, 11]. In Nigeria, the fruits are sometimes used in the treatment of piles and dysentery [12]. Phytochemical screening of the ethanolic extract of the fruits revealed the presence of alkaloids, tannins, saponins, phytates, flavonoids and cyanogenic glycosides, while proximate analysis revealed that the fruits are rich in proteins, fiber, carbohydrates, moisture, dry matter, vitamins A and C. The fruits were also shown to contain essential minerals such as Ca, Mg, K and P [13]. Isolation and characterization of some of the active constituents of the ripe and unripe fruits of the plants led to isolation of some phthalates [10, 11, 14] and fatty acid esters [15]. In view of the absence of much information on the phytoconstituents of the fruits, its ethyl acetate extract was further screened. This paper, therefore presents, the isolation and structural elucidation of some volatile constituents present in the ethyl acetate extract of the unripe fruits of *N. latifolia*.

### 2. Materials and Methods

**2.1 Collection of unripe fruits:** The unripe fruits of *N. latifolia* were collected from a farmland in Maikunkele area of Bosso Local Government area, Minna, Niger State, Nigeria, in the month of January, 2014. The fruits were authenticated by Mallam Gallah, of the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria and a voucher specimen was deposited.

**2.2 Extraction procedure:** Air-dried and pulverized unripe fruits of *N. latifolia* (500 g) were extracted with ethyl acetate by continuous extraction method until the extractant became colorless. The resulting solution was filtered, concentrated in vacuo and further dried over a water bath to afford a greenish-brown gummy mass, labeled ethyl acetate extract, E.

**2.3 Fractionation of crude ethyl acetate extract, E:** Five grams of extract E, was homogeneously mixed with petroleum ether (10 ml) and adsorbed onto silica gel (10 g). It was evaporated to dryness and the homogeneous mixture subjected to fractionation by flash column chromatography using silica gel (mesh 230-400, 100 g) and petroleum ether (60-80 °C) as stationary and mobile phases respectively. Gradient elution was carried out with varying proportions of petroleum ether: ethyl acetate. Eluents were monitored by TLC and identical fractions pooled. Fractions from solvent system petroleum ether: ethyl acetate (19:1) and petroleum ether: ethyl acetate (9:1) revealed promising spots. The two fractions were labeled E1 and E2 respectively.

**2.4 Purification of fraction E1:** TLC of fraction E1 using petroleum ether: chloroform (4:1) as the mobile phase revealed one major and two minor spots which were resolved by subjecting the fraction to further purification using flash chromatography (silica gel, mesh 230-400, 30 g). Elution was carried out with varying proportions of petroleum ether: chloroform. Elution with solvent mixture petroleum ether: chloroform (4:1) yielded a major single spot on TLC with some minor impurities at the origin. Concentration, drying and washing of the white crystals severally with methanol, afforded white crystalline flakes coded E1-A.

**2.5 Purification of fraction E2:** TLC of fraction E2 using petroleum ether: chloroform (2:1) as the mobile phase revealed 2 major and three minor spots which were resolved by subjecting the fraction to further purification using flash chromatography (silica gel, mesh 230-400, 30 g). Elution was carried out with varying proportions of petroleum ether and chloroform. Elution with solvent mixture petroleum ether: chloroform (1:1) yielded two major spots only on TLC that were resolved using preparative TLC (petroleum ether: ethyl acetate, 4: 1) to yield two different compounds, still with some impurities. The compounds were coded E2-A (a white waxy-like compound) and E2-B (white amorphous crystals). Further purification of both compounds by washing individually severally with methanol, afforded two pure isolates coded E2-A and E2-B.

**2.6 Thin layer chromatography (TLC):** TLC was carried out using oven baked pre-coated aluminum plates as the stationary phase and various solvent systems as the mobile phase. Chromatograms were viewed under (i) sunlight (ii) UV (254 and 366 nm) (iii) I<sub>2</sub> vapor and; sprayed with (iv) vanillin-sulphuric acid + heat and (v) 5% alc. KMnO<sub>4</sub>.

**2.7 Characterization of Isolates:** IR and UV were recorded both in CHCl<sub>3</sub> using FTIR (Spectrolab MB3000) and UV spectra (T60 UV-visible spectrophotometer) respectively, while, GC-MS was recorded using GCMS-QP 2010 plus Shimadzu. <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and DEPT-135 spectra were taken in CDCl<sub>3</sub> on JEOL LA spectrometer operating at 500MHz. Melting points were uncorrected.

### 3. Results and Discussion

#### 3.1 Physical and Spectral Characterization of the three Isolates

**3.1.1 Palmitic acid (Compound E1-A):** White crystalline flakes (21.4 mg), melting point, 61-64 °C, soluble in hexane and chloroform; insoluble in acetone, methanol and water. TLC, single spotted, R<sub>f</sub> 0.64 (no color in sunlight; UV active;

golden brown in iodine; blue with vanillin-sulphuric acid + heat and deep brown with 5% alc. KMnO<sub>4</sub>. GC-MS revealed its molecular formula and weight as C<sub>16</sub>H<sub>32</sub>O<sub>2</sub> and 256 gmol<sup>-1</sup> respectively.

IR (ν max, cm<sup>-1</sup>) 3465.38 (OH), 1709.27 (CO) 1488.74 (C-H in CH<sub>3</sub>) and 1021.66 (C-H in CH<sub>2</sub>)

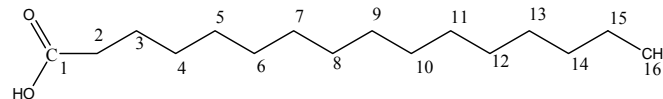
UV (λ max, nm) 147 (C-C, C-H bonds), 200 (COOH)

<sup>1</sup>H-NMR (δppm) 10.6 (d, H-1), 2.01 (t, H-2), 1.52 (m, H-3), 1.25 (sharp s, H-4 to H-14), 1.29 (m, H-15) and 0.95 (t, H-16)

<sup>13</sup>C-NMR analyzed with DEPT 135 (δppm) 175.2 (C-1), 34.5 (C-2), 22.8 (C-3), 27.4 (C-4), 27.6 (C-5), 27.8 (C-6 to C-12), 27.6 (C-13) 29.9 (C-14), 21.7 (C-15) and 13.8 (C-16); one quaternary (C-1), fourteen methylene (C-2 to C-15), one methyl (C-16) and absence of methine groups.

GC-MS (m/z peaks) 256[M<sup>+</sup>, C<sub>16</sub>H<sub>32</sub>O<sub>2</sub>]<sup>+</sup>, 213[C<sub>13</sub>H<sub>25</sub>O<sub>2</sub>]<sup>+</sup>, 129[C<sub>7</sub>H<sub>13</sub>O<sub>2</sub>]<sup>+</sup>, 98[C<sub>6</sub>H<sub>10</sub>O]<sup>+</sup>, 85[C<sub>4</sub>H<sub>5</sub>O<sub>2</sub>]<sup>+</sup>, 73[C<sub>3</sub>H<sub>5</sub>O<sub>2</sub>]<sup>+</sup>, 60[C<sub>2</sub>H<sub>4</sub>O<sub>2</sub>]<sup>+</sup>, 57[C<sub>4</sub>H<sub>9</sub>]<sup>+</sup>, 55[C<sub>4</sub>H<sub>7</sub>]<sup>+</sup>, 43[base peak, C<sub>3</sub>H<sub>6</sub>]<sup>+</sup> and 41[C<sub>3</sub>H<sub>5</sub>]<sup>+</sup>.

The IR and UV spectra showed characteristic bands indicating the presence of σ-σ\* bonds of C-C and C-H and carboxylic acid group. The proton NMR revealed a single sharp peak at δ1.25, an indication that several methylene groups were joined together in the same environment, while de-protonated carbon-13 showed that the compound is made up of sixteen carbon atoms. The most characteristic peak in the GC-MS spectra is m/z 60 which is due to the Mc-Lafferty rearrangement [16]. Other peaks are as a result of oxygen-containing fragment (213, 129, 98, 85 and 73) and alkyl-containing fragment (57, 55, 43 and 41). Compound E1-A, a non-polar fatty acid identified as Palmitic acid (Fig. 1) is a common fatty acid that has been reported and isolated from several medicinal plants [17, 18, 19].



**Fig 1:** Palmitic acid/Hexadecanoic acid/Pentadecanecarboxylic acid (a fatty acid).

**3.1.2 Ethyl palmitate (Compound E2-A):** White waxy-like (13.4 mg), melting point, 40-43 °C, soluble in chloroform; slightly soluble in hexane and acetone; insoluble in methanol and water. TLC, single spotted, R<sub>f</sub> 0.52 (no color in sunlight; UV active; golden brown in iodine; blue with vanillin-sulphuric acid + heat and deep brown with 5% alc. KMnO<sub>4</sub>. GC-MS revealed its molecular formula and weight as C<sub>18</sub>H<sub>36</sub>O<sub>2</sub> and 284 gmol<sup>-1</sup> respectively.

IR (ν max, cm<sup>-1</sup>) 2900-2850 (C-H, aliphatic), 1702.95 (CO) 1487.95 (C-H in CH<sub>3</sub>), 1300-1200 (CO, acetate) and 1014.82 (C-H in CH<sub>2</sub>)

UV (λ max, nm) 274 (CO)

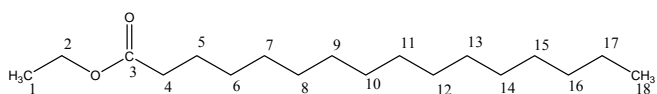
<sup>1</sup>H-NMR (δppm) 1.26 (t, H-1), 4.04 (q, H-2), 2.18 (t, H-4), 1.61 (q, H-5) 1.27 (sharp s, H-6 to H-16), 1.30 (m, H-17) and 0.95 (t, H-18)

<sup>13</sup>C-NMR analyzed with DEPT 135 (δppm) 13.8 (C-1), 60.5 (C-2), 171.6 (C-3), 31.7 (C-4), 24.3 (C-5), 28.4 (C-6), 28.9 (C-7), 29.1 (C-8 to C-14), 28.9 (C-15), 31.4 (C-16), 22.3 (C-17) and 14.0 (C-18); one quaternary (C-3), fifteen methylenes (C-2, C-4 to C-17), two methyl (C-2 and C-18) and no methine groups.

GC-MS (m/z peaks) 284[M<sup>+</sup>, C<sub>18</sub>H<sub>36</sub>O<sub>2</sub>]<sup>+</sup>, 239[M-OC<sub>2</sub>H<sub>5</sub>]<sup>+</sup>, 157[C<sub>9</sub>H<sub>17</sub>O<sub>2</sub>]<sup>+</sup>, 101[C<sub>5</sub>H<sub>9</sub>O<sub>2</sub>]<sup>+</sup>, 88[base peak, C<sub>4</sub>H<sub>8</sub>O<sub>2</sub>]<sup>+</sup>, 73[C<sub>3</sub>H<sub>5</sub>O<sub>2</sub>]<sup>+</sup>, 57[C<sub>4</sub>H<sub>9</sub>]<sup>+</sup>, 43[C<sub>3</sub>H<sub>6</sub>]<sup>+</sup>, 41[C<sub>3</sub>H<sub>5</sub>]<sup>+</sup>, 29 [C<sub>2</sub>H<sub>5</sub>]<sup>+</sup>, 27

$[C_2H_3]^+$ .

The IR and UV spectra showed characteristic bands indicating the presence of a carboxyl group of an ester. The proton NMR also revealed a single sharp peak at  $\delta$ 1.27, an indication that several methylene groups were joined together in the same environment, while de-protonated carbon-13 showed that the compound is made up of eighteen carbon atoms. GC-MS spectra gave a prominent peak at  $m/z$  239 indicating the loss of an alkoxy group from the molecular ion,  $M^+$ . This is typical of an ethyl ester. Characteristic peaks at  $m/z$  88 and 73 are due to Mc-Lafferty rearrangement, while peaks at 239, 157 and 101 and 57, 43, 41, 29 and 27 are due to oxygen- and alkyl-containing fragments respectively [16]. Compound E2-A, also a non-polar compound identified as Ethyl palmitate (Fig 2) is a common fatty acid ester that has been reported and isolated in several medicinal plants [17, 20, 21].



**Fig 2:** Ethyl palmitate/Palmitic acid, ethyl ester/Hexadecanoic acid, ethyl ester (a fatty acid ester)

**3.1.3. Ethenyl pentadecanoate (Compound E2-B):** White amorphous substance (22.8 mg); soluble in chloroform; slightly soluble in hexane and acetone; insoluble in methanol and water. TLC, single spotted,  $R_f$  0.41 (no color in sunlight; UV active; golden brown in iodine; blue with vanillin-sulphuric acid + heat and deep brown with 5% alc.  $KMnO_4$ . GC-MS revealed its molecular formula and weight as  $C_{17}H_{32}O_2$  and  $268 \text{ gmol}^{-1}$  respectively.

IR ( $\nu$  max,  $\text{cm}^{-1}$ ) 2900-2850 (C-H, aliphatic), 1702.95 (CO), 1612.93 (alkene), 1487.95 (C-H in  $CH_3$ ), 1300-1200 (CO, acetate) and 1014.82 (C-H in  $CH_2$ )

UV ( $\lambda$  max, nm) 274 (CO), 181 (alkene)

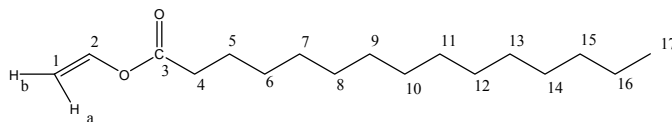
$^1H$ -NMR ( $\delta$ ppm) 7.28 (s, H-2), 4.53 (t, H-1a), 4.28 (t, H-1b), 2.39 (t, H-4) 1.67 (q, H-5), 1.54 (sharp s, H-6 to H-15), 1.47 (d, H-16), 0.96 (t, H-17)

$^{13}C$ -NMR analyzed with DEPT 135 ( $\delta$ ppm) 174.6 (C-3), 135.5 (C-2), 100.4 (C-1), 33.9 (C-4), 26.7 (C-5), 28.7 (C-6), 29.2 (C-7, C-14), 29.4 (strong, C-8 to C-13), 30.5 (C-15), 22.7 (C-16) and 14.0 (C-17); one quaternary (C-3), fourteen methylenes (C-1, C-4 to C-16), one methyl (C-17) and one methine (C-2) groups.

GC-MS ( $m/z$  peaks) 268 $[M^+$ ,  $C_{17}H_{32}O_2]^+$ , 225 $[M-OC_2H_3]^+$ , 197 $[M-C_{14}H_{29}]^+$ , 99 $[C_5H_7O_2]^+$ , 86 $[C_4H_6O_2]^+$ , 71 $[C_3H_5O_2]^+$ , 56 $[C_4H_8]^+$ , 55[base peak,  $C_4H_7]^+$ , 43 $[C_3H_6]^+$ , 41 $[C_3H_5]^+$ , 29 $[C_2H_5]^+$ , 27 $[C_2H_3]^+$

IR suggests it is an ester, while UV shows the compound possesses  $sp^2$  hybridized carbon atoms. Its Proton NMR suggests the presence of de-shielded protons of an allylic group (H-1 and H-2). Generally,  $\pi$  bonds are effective in influencing the chemical shift of nearby atoms, so that allylic H's are shifted downfield/higher  $\delta$  values [22]. The common peak at  $>\delta$ 170 for all the three is attributable to a quaternary carbonyl carbon. A carbonyl group exhibits anisotropic effects on adjacent atoms. This causes the carbonyl carbon and the hydrogen atoms bonded to them to resonate at the lowest field position because of the combined effects of the induced anisotropic field and a nearby electronegative element [22]. Its de-protonated carbon-13 spectra revealed that the compound was made up of seventeen carbon atoms, while its GC-MS spectra revealed a prominent peak at  $m/z$  225, a peak due to

the loss of  $OR^+$  group from the parent, M. Characteristic peak at  $m/z$  86 is due to Mc-Lafferty re-arrangement. Other peaks are as a result of oxygen-containing fragment (99, 86 and 71) and alkyl-containing fragment (56, 55, 43, 41, 29 and 27) [16]. Correlation of data obtained with literature values revealed almost similar data with ethenyl pentadecanoate (Fig 3). Ethenyl pentadecanoate has been reported and isolated from some medicinal plants [23, 24, 25, 26, 27, 28, 29, 30].



**Fig 3:** Ethenyl pentadecanoate/ Pentadecanoic acid, ethenyl ester/ Pentadecanoic acid, vinyl ester/ Vinyl pentadecanoate (a fatty acid ester)

#### 4. Conclusion

Purification of the crude chloroform extract of the unripe fruits of *N. latifolia* led to the isolation of three pure compounds. These compounds were identified and characterized based on spectroscopic data as Palmitic acid, Ethyl palmitate and Ethenyl pentadecanoate. This is the first report of isolation and characterization of all three compounds from the unripe fruits of *N. latifolia*.

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