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Original Research article

Isolation and Characterization of Fatty Acid Derivatives from the Stem Barks of *Albizia Amara* (Fabaceae), Sudanese Medicinal Plant

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ABSTRACT

In this work, four fatty acid derivatives including, methyl tritriacont-17-en-1-oate **1**, (9*Z*, 12*Z*)-ethyl octadeca-9,12-dienoate **2**, stearic acid **3,** and 21-acetyl tetracosanoic acid **4** were isolated from acetone extract of the stem barks of *Albizia amara* by chromatographic separation [TLC and CC]. The structures of the isolated compounds were established on the basis of extensive spectroscopic studies including 1D, 2D-NM, and MS analysis, and compared with literature. Compound **1**, **2** and **3** were reported first time from the *Albizia* genus and compound **4** was a new phytoconstituent isolated for the first time from plant sources.

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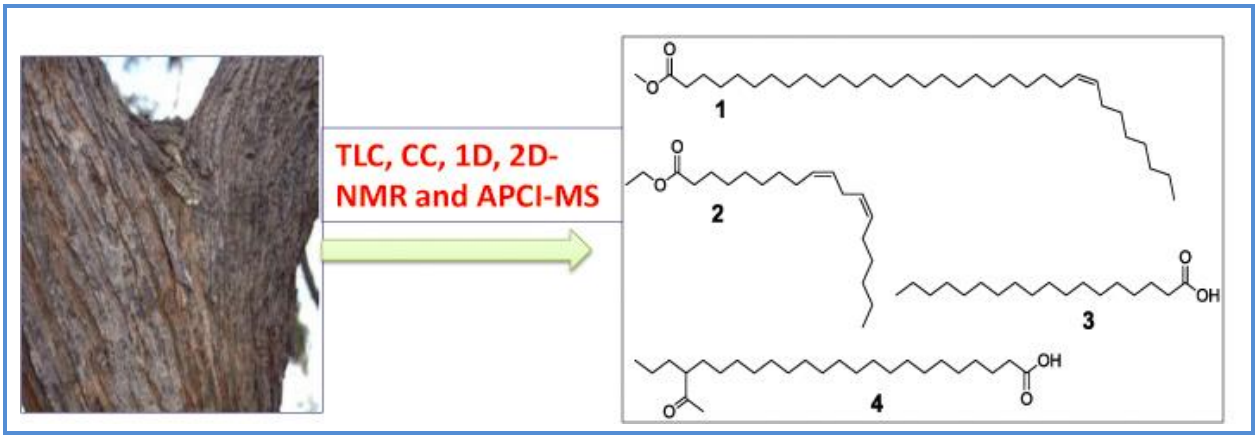
Graphical Abstract



Introduction

Fabaceae family is one of the 150 species available from the genus *Albizia* [1]. The mainly shrub and tree native to tropical and subtropical regions of Africa including Sudan, Kenya, Zambia, and Madagascar also found in many other parts of Asia, and North America [2, 3]. *A. amara* is the most important Sudanese traditional medicinal plants used for alleviating a variety of ailments, the aqueous leaf extracts of *A. amara* is use by traditional healers for treatment of diarrhea, epilepsy, severe backache, loin pain and other abdominal problems [4, 5]. Other medicinal uses of *A. amara* have also been reported from different parts of the world. In India and Tamilandu this plant is ethnomedically used for dandruff whereby the leaves previously dirtied in the shade are powdered and applied on the scalp [6]. The seeds of *A. amara* are considered as astringent and used in the treatment of diarrhea, piles, and gonorrhea. The flowers are used as a remedy for ulcer, cough, and dandruff [7]. The barks of the tree yield gum which is utilized for ulcer and molluscidal infection, also used as an astringent in diarrhea and dysentery and internally to check the uterine bleeding and the discharge in gonorrhea and topically in ophthalmia, and as a wound dressing [8-10]. *A. amara* has been reported to have anticancer [11-15], antimicrobial [16], antioxidant [17, 18] and anti-inflammatory properties [19]. Many studies are available on chemical constituents isolated from roots, stem barks, leaves and seeds of *A. amara* were terpenes, flavonoids, rare amino acids lipids and macrocyclic alkaloids [20, 21]. Our previous study on this plant has let to isolation and structural elucidation of two new phthalate derivatives [22]. In this study, we discussed the isolation and characterization of the fatty acid derivatives (Figure 1).

Experimental

General experimental procedure

NMR spectra were recorded using a Bruker-DRX-400-NMR, (^{1}H at 400 Hz and ^{13}C at 100 Hz) spectrometer (Bruker Biospin Inc, Germany) and chemical shift values were obtained on a δ (ppm) scale with TMS as internal standard. 2D-NMR experiment was performed using a standard Bruker micro-program (XWIN-NMR version 2.6 software). APCI-MS experiment was performed using micromass Q–TOF micro instrument, with an atmospheric pressure chemical ionization source. Column chromatography was carried out on silica gel (Merck kiesel gel 300-400 mech), TLC were carried out on silica gel GF254 (Merck), all the chemicals and solvents were commercial grade and used after further purification by simple distillation plant material.

The stem barks of *Albizia amara* were collected in February 2016 from the central Darfur state, west Sudan, and the plant was authenticated by prof. G.A. Yagoub, department of basic sciences, faculty of agriculture, university of Zalingei where a voucher specimen (No. 20171017) was deposited in the herbarium of author's laboratory.

Extraction and isolation methods

The barks of *Albizia amara* were air-dried for four weeks and grinded in to a powder. The bark powder (1 Kg) was extracted three times with acetone at room temperature (each 7 days×2 L). After evaporation of the solvent under vacuum (rotary evaporation), a dried residue (75 g) was obtained, which was subjected to column chromatography (13×100 cm) over silica gel (1000 g), the column eluted with pure $CHCl_3$ and EtOAc to yield **Fr1** (23 g) and **Fr2** (31 g) respectively. **Fr1** (23 g) was loaded on a silica gel (200 g), column (5×50 cm) eluting with PE:$CHCl_3$ (20:1-1:1), yielding thirteen fractions (A to M), then checked by TLC using PE: $CHCl_3$ (5:1--1:1) together to provided three Subfractions (**Fi**, **Fii** and **Fiii).** Subfraction (**Fi**) was further chromatographed again using PE - $CHCl_3$ (5:1) and recrystallized with mixed MeOH- $CHCl_3$ (0.5:1) to obtained compound **1.** Subfraction (**Fii**) was further purified on column chromatography used PE:$CHCl_3$ (3:1) to afford pure compound **2** while subfraction **(Fiii)** was subjected on CC eluted with PE; $CHCl_3$ gradient (2:1 and 1:1) elution to yielded compound **3**. Freaction **F2** (31 g) was subjected to column chromatography using PE:EtOAc (8:1-1:1) as the eluting system. Four fractions (**i** and **ii**) were obtained. Fraction **ii** was further subjected to column chromatography (CC) using PE:EtOAc (5:1) to give compound **4**.

Methyl tritriacont-25-en-1-oate 1: was obtained colourless solid, m.p. 78-80 °C, APCI-MS: 507.4326 $[M+H]^+$, $[C_{34}H_{66}O_2+H]^+$, ^{1}H NMR (400 MHz, $CDCl_3$), δ 5.34 (2H, dd, J = 11.3, 6.2 Hz, H-25, H-26), 3.48 (3H, s, H-1'), 2.34 (2H, t, J = 7.5 Hz, H-2), 2.03 (4H, m, H-24, H-27), 1.64 (2H, m, H-3), 1.30 (2H, m, H-31), 1.25-1.29 (40H, br s H-4 –H-23 and H-28, H-29, H-30), 0.88 (3H, t, J = 6.9 Hz, H-33). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (C-1) 179.56, (C-8) 130.00, (C-9) 129.69, (C-1') 50.79, (C-2) 33.93, (C-32) 31.88, (C-4 to C-24 and C-29, 30) 28.04 to 29.70, (C-26, 27) 27.13,27.19, (C-3) 24.67, (C-32) 22.65, (C-33) 14.07.

(9*Z*, 12*Z*)-ethyl octadeca-9,12-dienoate 2: was obtained colorless clear liquid. APCI-MS: 309.5045, $[C_{20}H_{36}O_2+H]^+$, ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.35 (4H, m, H-9, H-10, H-12 and H-13), 4.12 (2H, q, J = 7.1, CH_2-1'), 2.78 (2H, dd, J = 6.8 Hz, CH_2-11), 2.28 (2H, t, J = 7.6 Hz, CH_2-2), 2.03 (4H, m, CH_2-8, CH_2-14), 1.60 (4H, m, CH_2-7, CH_2-15), 1.27-1.30 (12H, m, CH_2-3 to CH_2-6 and CH_2-15 to CH_2-16), 1.13 (6H, t, J = 7.4, 6.9 Hz, CH_2-2', CH_2-18). ^{13}C-NMR (100 MHz, $CDCl_3$): δ (C-1) 173.86, (C-2) 31.89, (C-3) 25.61, (C-4 to C-7 and C-15, 16) 29.10-29.75, (C-9, 13) 30.06, (C-10, 12) 127.94, (C-8, 14) 27.17, (C-1') 60.12, (C-2', 18) 14.70, 14.23.

Stearic acid 3: was obtained as white solid, m.p. 70-72 °C, APCI-MS: 285.2191, $[M+H]^+$, $C_{18}H_{36}O_2$, ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.34 (2H, t, J = 7.5 Hz, H-2), 1.63 (2H, m, H-3), 1.28-1.30 (28H, m, H-4 to H-16), 0.88 (2H, t, J = 7.0 Hz, H-18), ^{13}C-NMR (100 MHz, $CDCl_3$): δ (C-1) 178.74, (C-2) 33.81, (C-3) 31.91, (C-4 to C-16) 29.7–30.00, (C-14) 31.91, (C-17) 22.68, (C-18) 14.11.

Compound 4: was obtained as white powder, m.p. 102-103 °C. APCI-MS fragment ions: *m/z* 410.248, 285.1382 and 257.2503. ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C-NMR (100 MHz, $CDCl_3$) see Table 1.

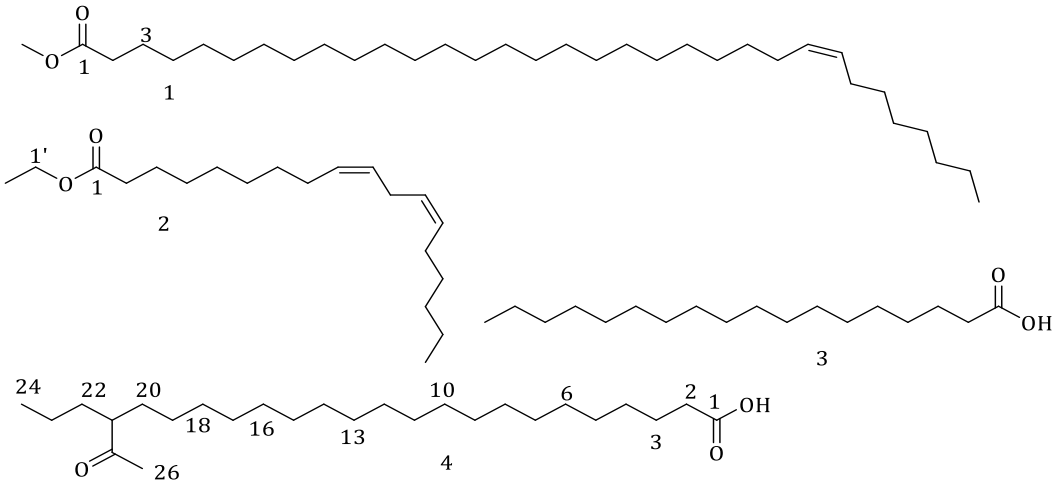


Figure 1. Structures of compounds **1**, **2**, **3**, and **4**

Results and discussion

Compound **1** was colourless solid, and the mass spectrum of this compound exhibited molecular ion at m/z 507 $[M+H]^+$ corresponding to the molecular formula of unsaturated aliphatic fatty acid methyl ester $C_{34}H_{66}O_2$, the most fragments were separated by 14 mass units that was indicated to straight chain nature of **1**. The ^{1}H NMR spectra of compound **1** exhibited a triplets at 0.88 δ with J =6.9 Hz each, representing three protons of a methyl group attached with methylene. Appearance of a broad peak at δ 1.30 suggested for 50 protons of 25 methylenes. A multiplet peak existing at δ 1.61 indicated for two protons of one methylene attached to methylene adjacent to alkoxy. A multiplet peak existing at 2.03 δ indicated for four protons of two methylenes attached to methines. A multiplet could be picked up from δ 2.34, denoted for two protons of $-CH_2COO$ group. A signal in the region of δ 3.48 signified three protons of methoxy group. A multiplet at δ 5.34, indicated for two proton of HC=CH group. The ^{13}C-NMR spectrum, exhibited signals for ester carbon at δ 179.05 (C-1), oxygenated methylene at δ 50.79 (C-1'). For the remaining 30 carbons, including two resolved peaks at δ C=129.69 and 130.0 corresponding to vinylic carbons and numerous overlapping signals in the region 29.04. 29.70 ppm was observed. The spectral and physical data were agreeable with those reported in the literature [23], which confirmed that the obtained compound **1** was methyl tritriacont-17-en-1-oate. This is first time reported compound from *Albizia amara.*

Compound **2** was obtained colourless liquid. Its APCI-MS data showed a molecular ion at *m/z* 309.50 $[M+H]^+$ corresponding to molecular formula $C_{20}H_{36}O_2$. The ^{1}H NMR spectrum showed vinylic protons as multiplets at δ 5.35 (m, 4H), one oxygenated methylene protons at δ 4.12 (2H, q, J = 7.1 Hz) was ascribed to oxygenated methylene protons CH_2-1'. Protons attached to the *bis*-allylic carbons at δ 2.78 ppm (2H, t, J = 6.8 Hz) and other methylene protons at δ 2.28 (2H, t, J = 7.6 Hz) was assigned to CH_2-2 methylene protons adjacent to the ester groups. A protons attached to the allylic carbons at δ 2.03 (4H, m), a methylene protons at δ 1.60 ppm (m, 4H). The other methylene protons appeared at δ 1.27 to 1.30 ppm (12 H, m) and primary methyl protons at δ 1.13 (6H, t, J = 7.4, 6.9 Hz). The ^{13}C NMR spectrum exhibited signals for ester carbon at δ 173.05 (C-1), vinylic carbons between 127.94-130.06, oxygenated methylene at δ 60.12 (C-2), other methylene carbons from δ 34.38 to 25.61 ppm and two methyl carbons at δ 14.23 (C-1') and 14.07 (C-18). The ^{1}H-^{1}H COSY spectrum of **2** revealed interactions of methyl protons H_3-1' with H_2-2' and H_3-18 with H_2-17, the double bonds 5.35 (H-10, H-12) correlated with methylenes CH2-11 at 2.78 ppm. The correlations from C-1 to C-16 clearly showed that the double bonds were located at C-9 and C-12.

The geometry of the double bonds was established by analyzing the ^{13}C NMR chemical shifts of the neighbouring carbons [24]. It is known that the carbons adjacent to *trans* double bonds have chemical shifts in the range of δ 29.5–38.0, whereas those adjacent to *cis* double bonds have values of δ 26.0–28.5. The ^{13}C NMR signals of compound **2** were at δ 27.17 (C-8) and 27.17 (C-13), confirming the percentage of *cis* double bonds between C-9 and C-10 as well as between C-12 and C-13. The HMBC showed cross beak between H-2, H-1'/C-1. The ester carbonyls at δ 173.86, showed 3*J* CH correlations with H-1' at δ 4.12, the methylene protons CH_2-1' at δ 4.12 showed connectivity to CH_3-2' at δ 14.23. The correlation of H-9 and H-13 at δ 5.35 revealed some connectivity with the C-8 and H-14 at d 27.17 of methylenes envelops. On the basis of above discussion and by comparing the spectral data with those reported in the literature [25], it was obtained that the compound **2** was (9*Z*, 12*Z*)-ethyl octadeca-9,12-dienoate.

Compound **3** was obtained as white solid, the mass spectrum of this compound exhibited molecular ion at *m/z* 285, $[M+H]^+$ corresponding to the molecular formula of saturated aliphatic fatty acid $C_{18}H_{36}O_2$, the most fragments were separated by 14 mass units that was indicated to straight chain nature of **3**. The ^1H NMR spectra of compound **3** in $CDCl_3$ exhibited a triplets at δ 0.88 δ with *J* =7.0 Hz. The spectrum indicated the presence of a long chain methylene protons representing 14 methylene groups overlapped as broad peak at δ 1.30, a multiplet peak existing at δ 1.63 indicated for two protons of one methylene attached to methylene adjacent to carboxyl group and a triplet peak at δ 2.34, denoted for two protons of $-CH_2COOH$ group. The ^{13}C NMR spectrum showed the presence of terminal methyl proton C-16 appeared at δ 14.11 attached to methylene carbon C-15 (δ 22.68). Another methylene carbon resonates at δ 31.91 (C-14), while methylene carbons C-13 to C-4 overlapped between δ 29.7–30.00. The existence of carbonyl signal was observed at δ 178.74. NMR spectral data were agreeable with those reported in the literature [26], which confirmed that the obtained compound **3** was therefore characterized as stearic acid.

Compound **4** was isolated as white powder, at 102-103 °C. The APCI-**MS** spectrum of this compound showed molecular ion at *m/z* 410 $[M+H]^+$, corresponding to the molecular formula $C_{26}H_{51}O_3$. The prominent ion fragment arising at *m/z* 285$[M+H]^+$ corresponding to the molecular formula of saturated aliphatic fatty acid $[C_{18}H_{36}O_2]^+$ and fragment ion at *m/z* 257, $[M-C_{10}H_{19}O]^+$ the most fragments were separated by 14 mass units that was indicated to straight chain nature of **4**. The ^1H NMR spectrum of compound **4**, Table 1. showed five signals resonating at δ 0.87 triplet with *J*=7.0 Hz, contained three protons which were accounted to H-24 primery methyl protons. One large singlet peak at δ 1.29 (38H) was measured for 19 methylene (CH_2) groups, a multiplet peak existing at δ 1.63 indicated for two protons of one methylene($-CH_2$) attached to methylene adjacent to

carboxyl group and a triplet peak at δ 2.34, denoted for two protons of $-CH_2COOH$ group. A very important signal at 2.17 (3H) was indication of the presence of an acetyl protons, which were also confirmed through ^{13}C NMR by observing signals at δ 207.14 (C-21) and the existence of carbonyl signal was observed at δ 178.85 (C-1), the terminal methyl carbon C-24 appeared at δ 14.09 attached to methylene carbon C-23 (δ 22.67). Another methylene carbon resonates at δ 31.90 (C-19), while methylene carbons C-18 to C-4 overlapped between δ 29.05–29.66 pp, Table 1. The analysis of COSY and HMBC experiments (Figure 2) showed the correlation between methyl proton at δ 0.87 (3H, t, J = 6.8 Hz, H-24) and C-23 (δc 22.67), C-22 (δc 31.90). The correlation between the H-atom signal at δ 2.17 (3H, s, H-26) and C-25 (δc 207.14), C-21 (δc 50.66). The carboxylic carbon at δ 178.85 (C-1) revealed the correlations with H-2 at δ 2.34 (2H, J = 7.5 Hz, H-2) wich indicated the presence of aliphatic chain on the other side of molecule. According to the above information the structure of compound **4** was elucidated as 21-acetyltetracosanoic acid.

Table 1. ^{1}H- and ^{13}C-NMR spectral data of compound **4** in $CDCl_3$

Position	δ_C, 1	δ_H, 1
C-25	207.14	-
C-1	178.85	-
C-26	29.20	2.17 (3H, s)
C-2 and C-21	33.86 and 50.66	2.34 (2H, t, J = 7.5 Hz)
C-3, C-20 and C-22	31.90	1.63 (6H, *m*)
C-4 to C-19	29.05 to 29.66	1.29 (24H, br, s)
C-23	22.67	1.29 (2H br, s)
C-24	14.09	0.87 (3H, t, J = 6.8 Hz)

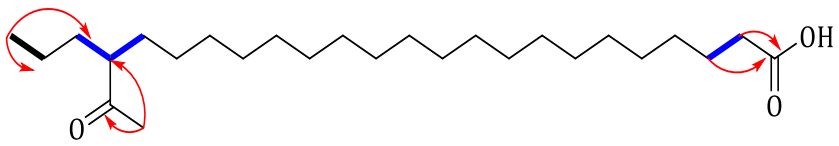


Figure 2. The key HMBC (⟶) and Cosy (—) correlations observed in compound **4**

Conclusions

The present study aim to describe the isolation and characterization of fatty acid derivatives present in acetone extract of the stem barks of *Albizia amara.* A new fatty acid derivative, 21-acetyltetracosanoic acid **4** was isolated together with three known compounds **1**, **2** and **3**. Their structures were elucidated by extensive spectroscopic methods including 1D (^{1}H and ^{13}C), 2D-NMR experiments, (1H-1H COSY and HBMC) and APCI-MS analysis. The existence of these bioactive

chemical in this plant might be attributed to the folk utilization of *A. Amara* for various ailment by traditional medical practitioners.

Conflict of Interest

We have no conflicts of interest to disclose.

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