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Fatty Acids and Its Derivatives from the Extracts of *Ficus mucoso* Leaves

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Abstract

The n-hexane, ethyl acetate and methanol extracts of *Ficus mucoso* leaves were screened for the presence of metabolites, using column and thin-layer chromatographic techniques. Dodec-6-enoic acid (1), methyl hexadecanoate (2), hexadec-9-enoic acid (3), and ethyl tetradecanoate (4) were obtained from the extracts of *F. mucoso* leaves. These compounds were confirmed via 1H and 13C Nuclear Magnetic Resonance Spectroscopy (NMR) and available published literature.

Keywords: *Ficus mucoso*; phytochemical isolation; plant metabolites; column and thin-layer chromatography; NMR spectroscopy.

INTRODUCTION

Medicinal plants are outstanding sources of therapeutic and pharmacological phytochemicals which may lead to the improvement of novel and efficient drugs and are of considerable significance (Hosseini and Ghorbani, 2015). Most of the phytochemicals from plant sources have been reported to possess pharmacological properties against acute diseases (Srujana et al., 2012), and in the prevention of deadly diseases such as cancer (Venugopal and Liu, 2012). The increasing interests in research and industrial applications of medicinal plants as alternative to conventional drugs and synthetic products, have contributed to the development and formulation of some cosmetic products and novel drugs (Mukherjee et al., 2011; Hosseinzadeh et al., 2015).

Ficus mucoso is a member of the family Moraceae (Berg, 1990). It is a large tree of about 40 m high, often buttressed. Its leaves are elliptic to almost round, 6–17 by 4–15 cm, sandpapery above, short-hairy beneath. *F. mucoso* is used in herbal medicine to treat cancer, diarrhoea, oedema, insanity, microbial infections and leprosy (Cokera and Adeniyi-Aogo, 2021). Its bark and leaf decoction are drunk for diarrhoea (Burkill, 1997; Somashekhar et al., 2013) and its latex, given to children with convulsion and instilled into the ear for otitis (Somashekhar et al., 2013; Bouquet, 1969). Some species of Ficus mucoso are used as foods and medicines in China and Cameroon (Bankeu et al., 2010).

Despite the ethnomedicinal uses of these plants, less attention has been paid to their exploitation. This study was designed to isolate (using column chromatography) and to characterize the constituents of *F. mucoso* extracts using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy.

MATERIALS AND METHODS

Chemicals and reagents

n-hexane, ethyl acetate, and ethanol were used in this study. Silical gel of mesh sizes 60/120, and 100/200, glass column, beaker, conical flask, iodine chamber, test tubes, precoated Aluminum and glass thin layer chromatographic plate, TLC chamber, shimadzu UV-1650PC spectrophotometer, etc. NMR spectra were recorded on Bruker 300 MHz NMR Spectrometers, ^{13}C-NMR spectra were recorded at 75 MHz. The proton resonances are annotated as chemical shifts δ parts per million (ppm) relative to tetramethylsilane (δ 0.0) using the residual solvent signal as an internal standard or tetramethylsilane itself: chloroform-d (δ 7.26, singlet), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad), coupling constant J, in hertz (Hz), and the number of protons for a given resonance indicated by nH. The chemical shifts of ^{13}C NMR are reported in ppm, relative to the central line of the triplet at δ 77.0 ppm for $CDCl_3$.

It is worthy of note that the isolated compounds has already been reported previously in literatures, although they are newly identified and first mentioned, in the current plants under investigation. And since the attached NMR spectral matches those cited in literatures, we did not proceed with mass spectrometry. However, we have cited papers where the molecular weights or MS values have been reported for clarity.

Plant Materials

Fresh leaves of *F. mucoso* were collected in Ilorin, Kwara State, Nigeria. The plants were identified and authenticated at the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria by Mr Bolu Ajayi (a botanist), and voucher specimens of the plants namely *F. mucoso* (UILH/004/1234) were deposited in the Herbarium of the department.

Extraction and Isolation Procedures

Ficus mucoso leaves were air-dried for three weeks and ground into powdery form with machine. The weights obtained after grinding was 606.28 g. The ground samples were soaked with n-hexane, ethyl acetate and methanol successively for 10 days each in the order of their polarity, for complete extraction. The extracts were filtered separately with Whatmann No.1 filter paper and concentrated at 45 ºC, using rotatory evaporator and then freeze-dried to remove trace solvents. The dried extracts were subjected to column chromatography to obtain pure isolates, and thin layer chromatography.

RESULTS AND DISCUSSIONS

Results

Extraction and Isolation

Extraction of *F. mucoso* leaves with n-hexane, ethyl acetate and methanol gave 21 g, 19 g, and 40 g extracts respectively. The methanol extract (40 g) of *Ficus mucoso* leaves (FMM), was pre-adsorbed on silica gel (60-200 mesh size) (60 g) to form powdered homogeneous slurry. The slurry was then subjected to column chromatography, packed using the slurry method (silica gel 500 g, 60-120 mesh size) and eluted with various solvent systems, starting with 100% of hexane; then using hexane: ethyl acetate (99:1, 100 mL); (98:2, 100 mL); (95:5, 100 mL); (93:7, 100 mL); (90:10, 100 mL); (85:15, 100 mL); (80:20, 100 mL); (75:35, 100 mL); (60:40, 100 mL); (55:45, 100 mL); (50:50, 100 mL); (45:55, 100 mL); (40:60, 100 mL); (30:70, 100 mL); (25:75, 100 mL); (20:80, 100 mL); (10:90, 100 mL); (5:95, 100 mL), ethyl acetate (100 %, 100 mL), and ethyl acetate : methanol (98:2, 100 mL), (96:4, 100 mL), (95:5, 100 mL), (94:6, 100 mL), and (92:8, 100 mL). A total of 40 fractions from the methanol extract were collected and pooled to two pure fractions, coded FMM-1 (18 mg) and FMM-2 (15 mg), based on TLC analysis. Finally, the ethyl acetate-hexane extract (19 g) of *F. mucoso* (FMEH) was subjected to chromatography following the procedures for above, and two pure fractions, coded FMEH-1 (13 mg) and FMEH-2 (16 mg), were obtained based on TLC analysis.

The Proton and Carbon-13 NMR Spectroscopy Results

Characterization of Dodec-6-enoic acid (Figure S1_FMM-1), $C_{12}H_{22}O_2$, white oily solid (18 mg); **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 0.68–1.0 (3H, t, H_{-12}, C**H_3**), 1.02–1.48 (8H, m, H_{-4} & $H_{-9–11}$, 5 × CH_2), 1.51–2.20 (4H, m, $H_{-5\ \&\ 8}$, 2 × CH_2), 5.30–5.57 (2H, m, H_{-6-7}, 2 × CH), 2.22–2.36 (4H, H_{-2} (t), O=C**CH_2** & H_{-3} (m)); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 179.44 (COOH, C_{-1}), 34.02 (C_{-2}), 29.71 ($C_{-3–5,\ 8–9}$), 130.04 (C_{-6}), 128.46 (C_{-7}), 29.10 (C_{-10}), 22.71 (C_{-11}), 14.14 (C_{-12}).

Characterization of Methyl hexadecanoate (Figure S2_FMM-2), $C_{17}H_{34}O_2$, yellow solid (22 mg); **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 0.88 (3H, t, H_{-16}, CH_3), 1.25–1.43 (24H, m, $H_{-4–15}$, 12 × CH_2), 2.17–2.37 (4H, H_{-2} (t) & H_{-3} (m), 2 × CH_2), 3.68 (3H, s, O**CH_3**); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 174.5 (C=O, C_{-1}), 34.46 (C_{-2}), 31.96 (C_{-3}), 29.73 (C_{-4}), 29.40 ($C_{-5–9}$), 29.29 (C_{-10}), 29.20 (C_{-11}), 28.69 (C_{-12}), 25.95 (C_{-13}), 25.07 (C_{-14}), 22.73 (C_{-15}), 14.15 (C_{-16}), 64.44 (O**C**H_3).

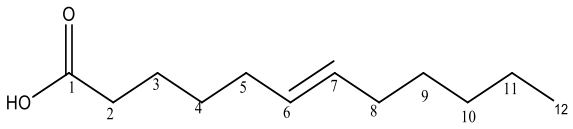
Characterization of Hexadec-9-enoic acid (Figure S3_FMEH-1), $C_{16}H_{30}O_2$, yellow viscous liquid (13 mg); **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 2.03–2.36 (4H, H_{-2} (t) & H_{-3} (m), 2 × CH_2), 1.25–1.33 (16H, m, $H_{-4–7\ \&\ 12–15}$, 8 × CH_2), 1.60–1.65 (4H, m, $H_{-8\ \&\ 11}$, 2 × CH_2), 4.98–5.58 (2H, m, $H_{-9–10}$, 2 × CH), 0.88 (3H, t, H_{-16}, CH_3); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 179.80 (COOH, C_{-1}), 37.46 (C_{-2}), 33.92 (C_{-3}), 32.82 (C_{-4}), 31.45 (C_{-5}), 30.06 (C_{-6}), 29.38(C_{-7}), 29.71 (C_{-8}), 128.32 (C_{-9}), 136.23 (C_{-10}), 29.47 (C_{-11}), 29.38 (C_{-12}), 24.58 (C_{-13}), 22.71 (C_{-14}), 19.78 (C_{-15}), 14.14 (C_{-16}).

Characterization of Ethyl tetradecanoate (Figure S4_FMEH-2), $C_{16}H_{32}O_2$, cream solid (16 mg); **^{1}H NMR ($CDCl_3$, 300 MHz):** δ 2.03–2.37 (4H, H_{-2} (t) & H_{-3} (m), 2 × CH_2), 1.11–1.39 (20H, m, $H_{-4–13}$, 10 × CH_2), 0.88 (3H, t, H_{-14}, CH_3), 4.05 (2H, q, $OC\mathbf{H_2}CH_3$ & 3H, t, $OCH_2C\mathbf{H_3}$); **^{13}C NMR ($CDCl_3$, 75 MHz):** δ 174.07 (C=O, C_{-1}), 34.46 (C_{-2}), 31.96 (C_{-3}), 29.73 (C_{-4}), 29.51 (C_{-5}), 29.39 (C_{-6}), 29.30 (C_{-7}), 29.20 (C_{-8}), 28,69 (C_{-9}), 25.97 (C_{-10}), 25.59 (C_{-11}), 25.07 (C_{-12}), 22.72 (C_{-13}), 14.15 (C_{-14}), 64.44 ($O\mathbf{C}H_2\mathbf{C}H_3$).

Discussions

The isolates from each of the plant extracts were characterized using ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) Spectroscopy. The ^{1}H and ^{13}C NMR analyses of the isolates obtained from the methanol extracts of *F. mucoso* leaves gave compounds **1** and **2** while the analyses on the isolates of ethyl acetate-hexane extract of *F. mucuso* leaves, gave compounds **3** and **4**. The structures of these compounds are shown in Figure 1.

Compound 1 (Fig. S1_FMM-1): 18 mg was obtained as white oily solid. The ^{1}H NMR spectrum of compound **6** showed peak at δ_H 0.68–1.0 (3H, t, H_{-12}) corresponding to the terminal methyl hydrogen atoms while the multiplet peaks at δ_H (1.02–1.48) were assigned to cluster of methylene hydrogen atoms at 4 & 9–11 positions. Allylic protons were shown by the presence of δ_H 1.51–1.63 and 2.20 at positions 8 & 5. Olefinic hydrogen atoms were shown by the presence of δ_H 5.30 & 5.57 respectively at C_{-6} & C_{-7}. The signal δ_H 1.69 & 2.22 correspond to α and β carbonyl hydrogen atoms at positions2 & 3. The ^{13}C NMR spectrum indicated twelve carbon resonances, sorted out as two olefinic carbons (δ_c 128.46, C_{-7} and 130.04, C_{-6}), carbonyl carbon (δ_C 179.44, C_{-1}), eight methylene carbons, and one methyl carbon (δ_c 14.14 (C_{-12})). The spectroscopic data of compound **1** were similar to those reported on dodec-6-enoic acid in the literature (Lie Ken Jie and Lam, 1995; Hamid et al., 2017a). Hence, the structure of compound **1** was established as Dodec-6-enoic acid.



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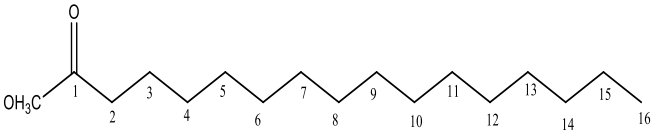
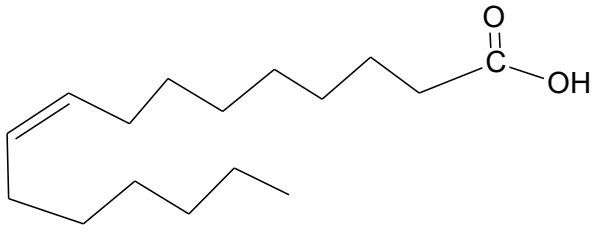
Compound 2 (Fig. S2_FMM-2): 22 mg was isolated as yellow crystals. The ^{1}H NMR spectrum showed peak at δ_H 0.88 (3H, t, H-16) correspond to terminal methyl proton while the multiplet peaks at δ_H 1.25–1.43 were assigned to cluster of methylene protons at 4–15 position. The ^{13}C NMR spectrum displayed seventeen carbon resonances and were sorted out as fourteen methylene carbons (δ_c 22.73–34.46, C_{-2} to C_{-15}), one methyl carbon (δ_c 14.15 (C_{-16})), oxymethyl carbon (δ_c 64.44, OCH_3) and a carbonyl carbon (δ_c 174.5, C_{-1}). The spectroscopic data of compound **2** were similar to those obtained on methyl hexadecanoate in the literature (Lie Ken Jie and Lam, 1995; Credo et al., 2022). Hence, the structure of compound **2** was established as methyl hexadecanoate.



2

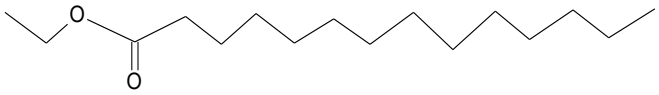
Compound 3 (Fig. S3_FMEH-1):13 mg) was isolated as a yellow viscous liquid. The ^{1}H NMR spectrum showed signals at δ_H 0.88 correspond to the methyl hydrogen atoms. The peaks at δ_H 1.25–1.33 correspond to the cluster of methylene hydrogen atoms. The signals at δ_H 1.60–1.65 correspond to the allylic hydrogen atoms. The peaks at δ_H 2.03–2.36 correspond to the methylene hydrogen atoms after the carbonyl carbon. The peaks at δ_H 4.98–5.58 correspond to the olefinic hydrogen atoms. The ^{13}C NMR spectrum showed 16 carbon resonances. The peaks at δ_c 179.80 correspond to the carboxyl carbon. The peak at δ_c 14.14 corresponds to the methyl carbon. The peaks at δ_c 128.32 and 136.23 correspond to the olefinic carbons, and the peaks at δ_c 19.78–37.46 correspond to the cluster of methylene carbons. The spectroscopic data of compound **3** were similar to those obtained in the literature (Hamid et al., 2017b; Ogunleye et al., 1991) for Hexadec-9-enoic acid. Therefore, the structure of compound **3** was established as Hexadec-9-enoic acid.



3

Compound 4 (Fig. S4_FMEH-2): 16 mg was isolated as a cream solid. The ^{1}H NMR spectrum showed peaks at δ_H 0.88 corresponding to the methyl hydrogen atoms. The peaks at δ_H 1.11–1.39 correspond to the cluster of methylene hydrogen atoms. The peaks at δ_H 2.03–2.37 correspond to the methylene hydrogen atoms next to the carbonyl carbon. The peaks at 4.05 correspond to the oxyethylene hydrogen atoms. The ^{13}C NMR spectrum showed 16 carbon resonances. The peak at δ_c 14.15 corresponds to the methyl carbon. The peaks at δ_c 174.07 correspond to carbonyl carbon. The peaks at 64.44 correspond to the oxyethylene carbons. The peaks at 22.72–34.46 correspond to the cluster of methylene carbons. The spectroscopic data of compound **4** were similar to those obtained for ethyl tetradecanoate in the literature (Credo et al., 2022; Hamid et al., 2019; Chuah

et al., 2006). Therefore, the structure of compound **4** was established as Ethyl tetradecanoate.



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CONCLUSION

Dodec-6-enoic acid (**1**) and methyl hexadecanoate (**2**) were isolated from the methanol extract of *Ficus mucoso* leaves, while hexadec-9-enoic acid (**3**) and ethyl tetradecanoate (**4**) were isolated from the ethyl acetate-hexane extract of *F. mucoso* leaves. These compounds are being reported for the first time from these plants.

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Disclosure statement: The authors declare that they have no competing interests.

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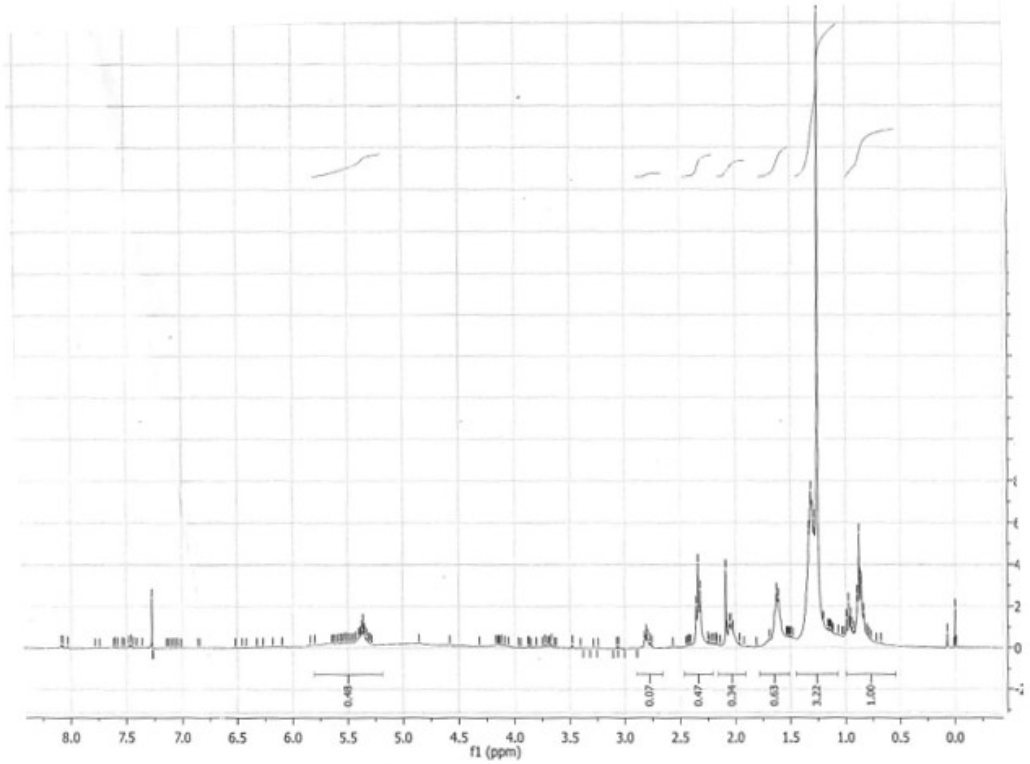

Figure S1a. [1]H NMR of Compound 1 (from *F. mucoso*).

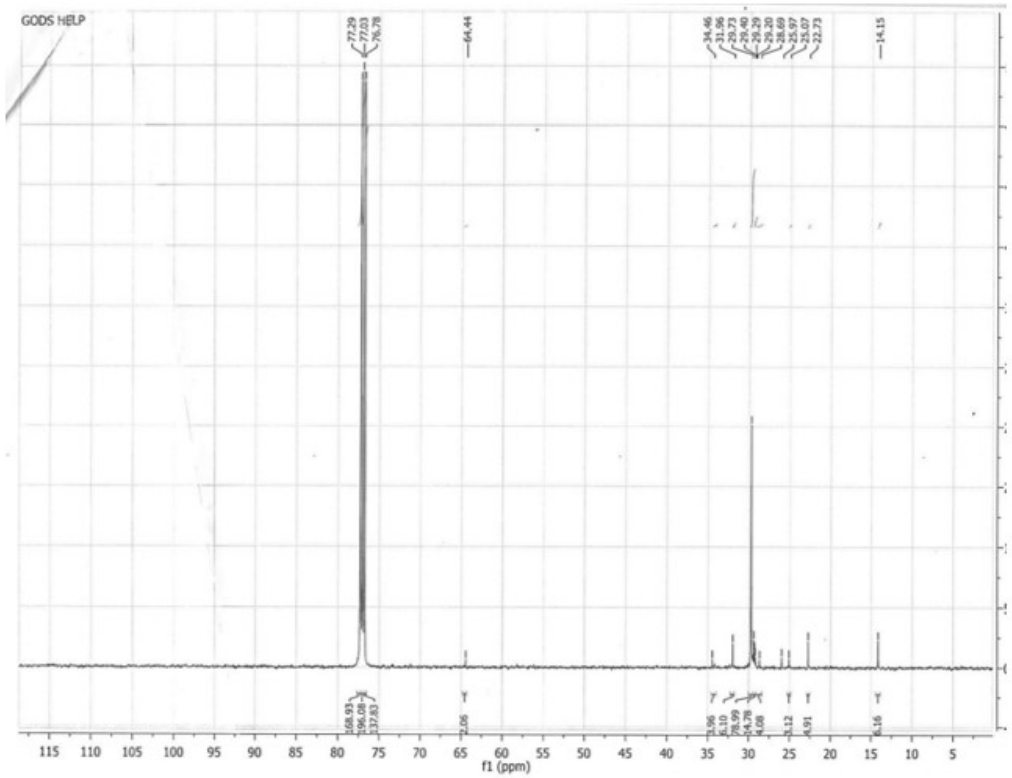

Figure S1b. [13]C NMR of Compound 1 (from *F. mucoso*).

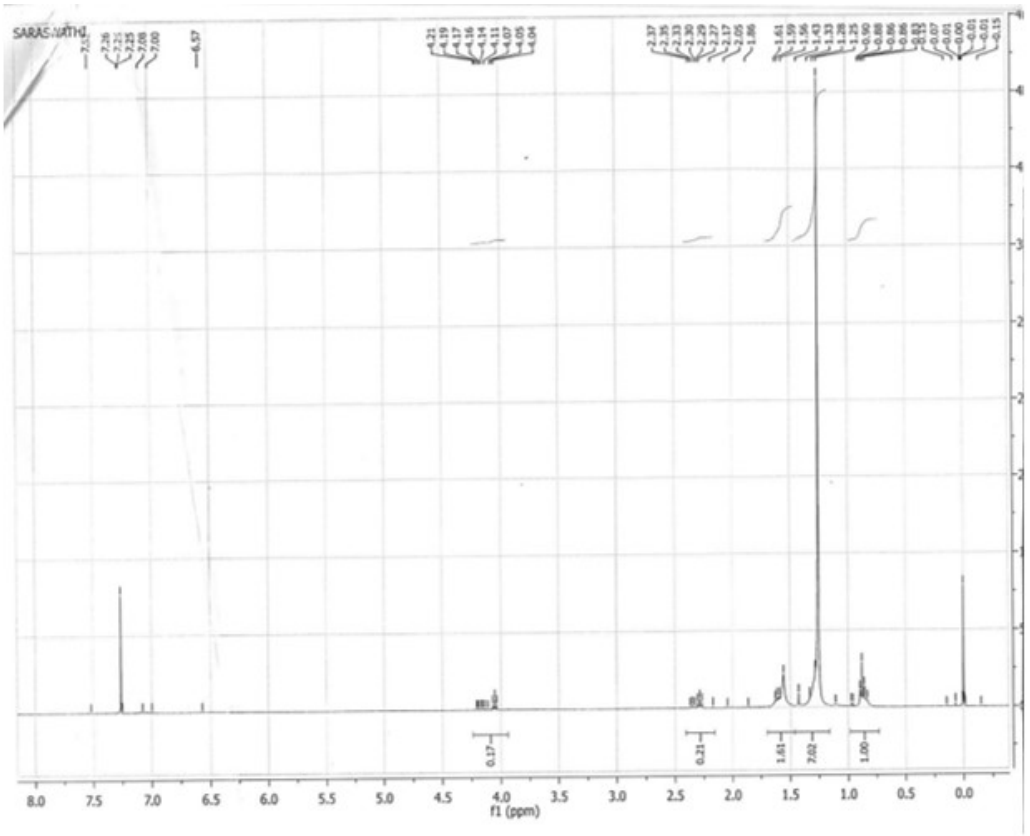

Figure S2a. [1]H NMR of Compound 2 (from *F. mucoso*).

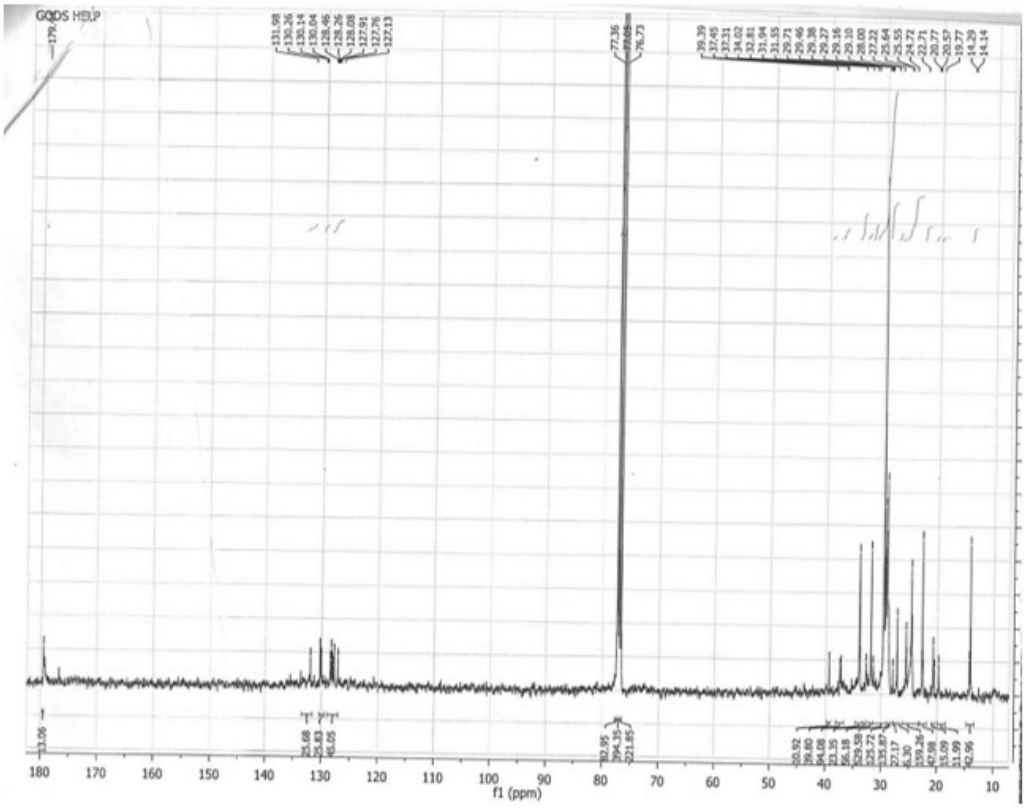

Figure S2b. [13]C NMR of Compound 2 (from *F. mucoso*).

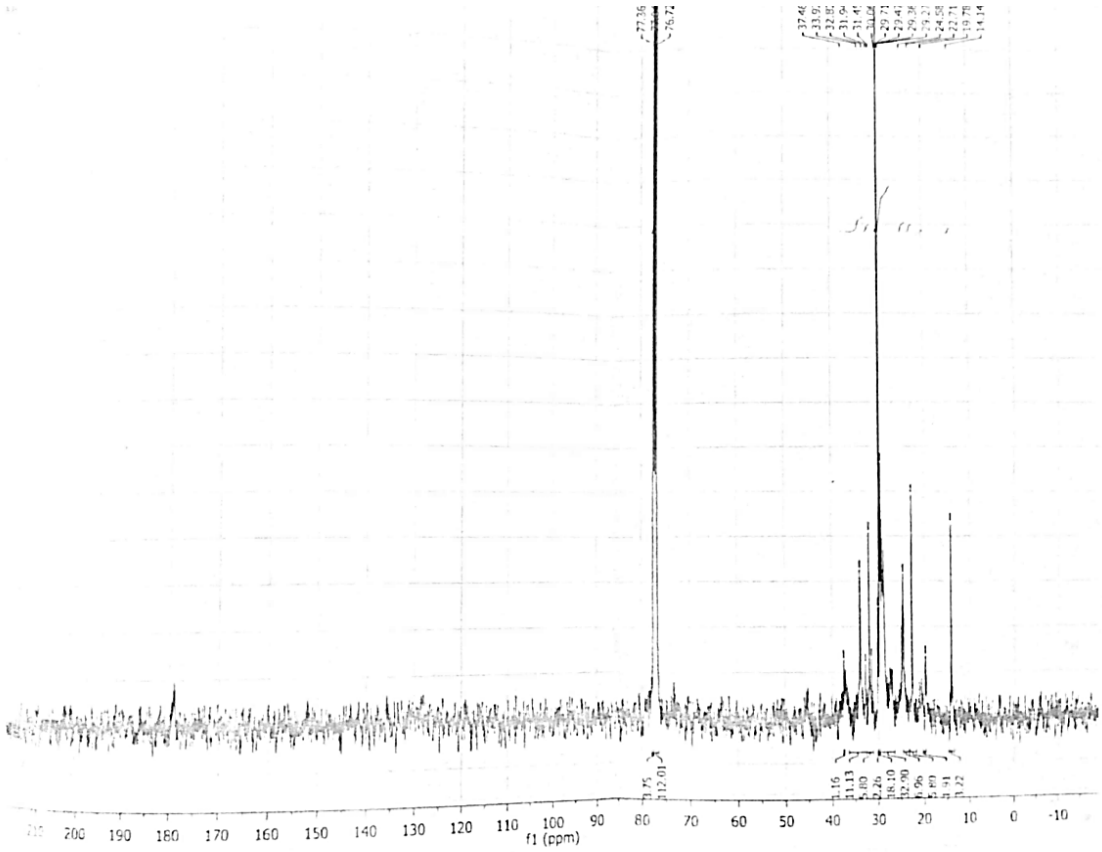

Figure S3. ^{13}C NMR of Compound 3 (from *F. mucoso*).

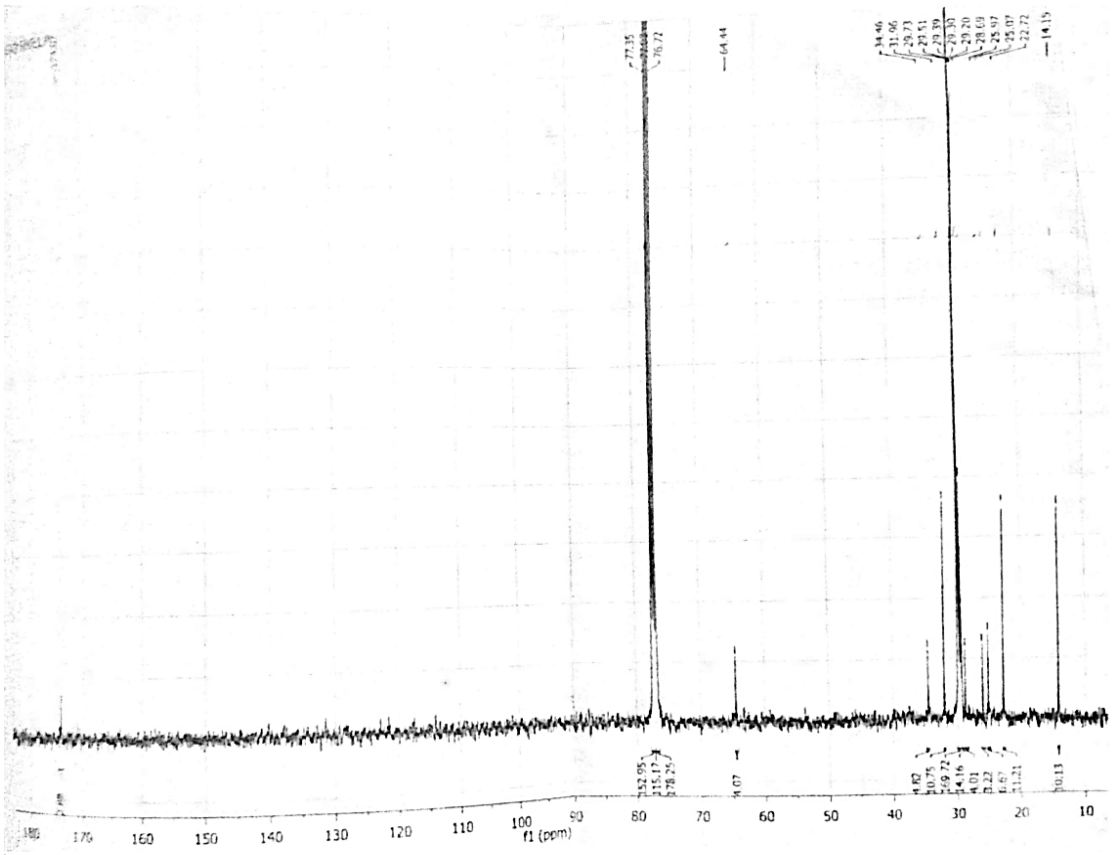

Figure S4. ^{13}C NMR of Compound 4 (from *F. mucoso*).

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