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Isolation and Partial Characterization of 11-Octadecenoic Acid Methyl Ester and Bis (2-ethylhexyl) Phthalate from *Celtis integrifolia* Lam

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Authors' contributions

This work was carried out in collaboration between both authors. Research conceptualization, design, manuscript drafting and editing was carried out by author KA while the experimental work and data collation was jointly done by authors MI and KA. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Celtis integrifolia* Lam is a traditional medicinal plant used as a remedy for the cure of diarrhea, measles, bleeding, ecbolic, sore throat and as an antinociceptive agent.
Aim: The study was aimed at the isolation and partial characterization of phytocompounds from *Celtis integrifolia* crude extract.
Methodology: The stembark sample was powdered and extracted using maceration technique with methanol. The extract was filtered using cotton wool and clarified using Whatmann No.1 filter paper. The filtrate was concentrated on a rotavapor at 45^{o}C. The crude extract was then reconstituted in water and partitioned successively with n-hexane, ethyl acetate, and n-butanol. The ethyl acetate and the n-hexane fractions were concentrated and subjected to purification on column chromatography.

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Results: Gradient elution of extract fractions on open-column chromatography yielded two compounds coded CiL1 and CiL2 from the n-hexane and ethyl acetate fractions respectively. The isolated compounds were characterized using spectroscopic data from Fourier transform infrared (FT-IR) spectroscopy, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS) and literature database. Compound CiL1 with R_f = 0.43 in hexane: Ethyl acetate (9:1) was identified as 11-Octadecenoic acid methyl ester while Compound CiL2 isolated from ethyl acetate fraction as a yellow substance with R_f = 0.38 in hexane: ethyl acetate (8:2) was identified as bis (2-ethylhexyl) phthalate.
Conclusion: The study showed the presence of 11-octadecenoic acid methyl ester and bis (2-ethylhexyl) phthalate in the stembark extract of *Celtis integrifolia* Lam. These two phytocompounds are reported for the first time from *Celtis integrifolia* Lam based on available literature. Since the presence of phytochemical substances is believed to be the basis of any observed pharmacological activity, the study therefore lends credence to the traditional medicinal uses of *Celtis integrifolia* Lam.

Keywords: Celtis integrifolia; 11-octadecenoic acid methyl ester; bis (2-ethylhexyl) phthalate; chromatography; characterization.

1. INTRODUCTION

Medicinal plant exhibits a broad range of biological activities due to the presence of diverse range of bioactive molecules with beneficial therapeutic properties [1]. Plants are considered as a reservoir of bioactive compounds which are useful in drug development and synthesis. These bioactive compounds had been exploited for the treatment of ailments and includes substances such as coumarins, terpenoids**,** carotenoid alkaloids, saponins, phenols, steroids, tannins and glycosides [2]. The *Celtis* genus is famous for the widespread use of its species in traditional medicine with several compounds isolated and characterized [3]. Specifically, Filali-Ansari et al*.* [4] reported the isolation and characterization of three antioxidant compounds from *Celtis australis* and were identified as β-sitosterol (1), β-sitosterol-3-O-β-glucoside (2) and vanillic acid (3).

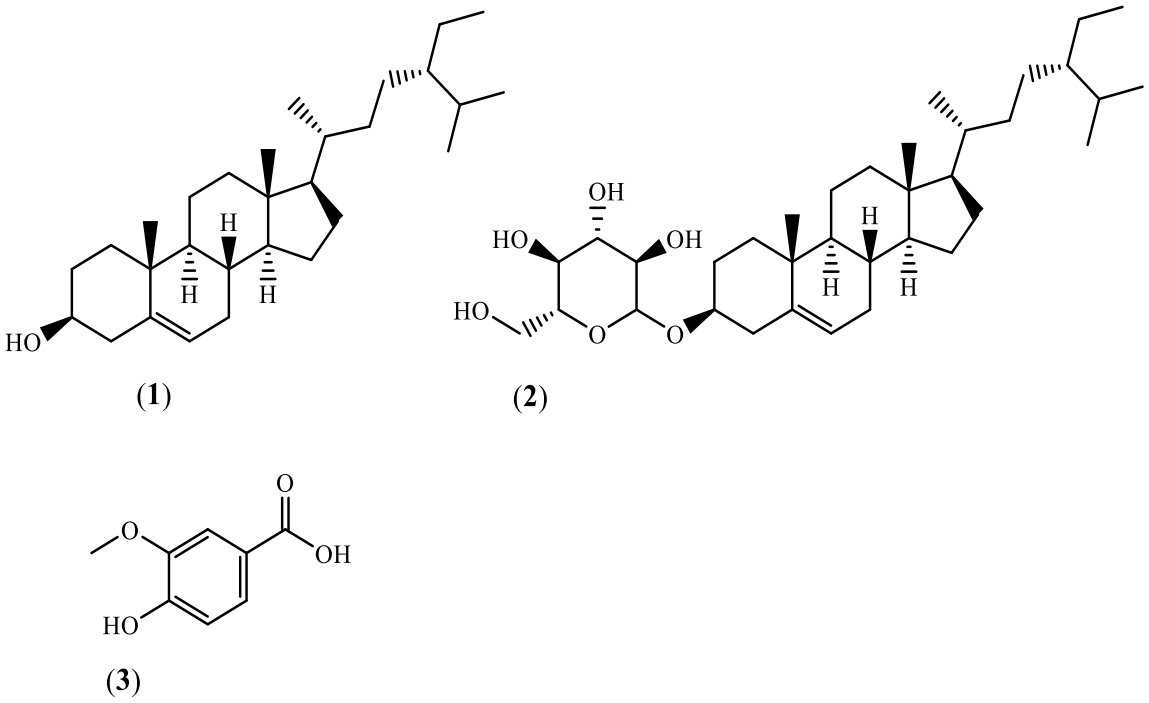

Image 1. Some compounds isolated from *Celtis australis*

Celtis integrifolia (syn*. Celtis toka*) is a deciduous tree that grows up to about 30 m tall usually with grey smooth stem bark. The plant is common to the temperate regions of the northern hemisphere and widely used as medicine in African countries like Nigeria. The leaves and the stem bark of the plant are reportedly the most used parts in traditional medicine for the treatment of epilepsy, mental disorders, cancer, wound healing, diarrhea, chicken pox, measles, bleeding, gout, ecbolic, sore throat and as an antinociceptive [5,6]. Recent studies had proved that *Celtis integrifolia* leaf extract contains several classes of phytocompounds such as alkaloids, phenols, steroids and flavonoids [7]. Despite several reported pharmacological properties such as antimicrobial, cytotoxicity and antioxidants activities [8,6] on *Celtis integrifolia* crude extract, there are no studies yet on the isolation and characterization of its phytocompounds based on available literature. Consequently, we report for the first time the isolation and partial characterization of 11-octadecenoic acid methyl ester and bis (2-ethylhexyl) phthalate from C. integrifolia Lam.

2. MATERIALS AND METHODS

2.1 Collection and Sample Identification

The stembark sample of *Celtis integrifolia* Lam was collected from NGalda, Yobe State Nigeria in December 2022 and identified by Dr. D. A. Zhigila Department of Botany, Gombe State University. The sample was compared with a previously deposited specimen and Voucher No. GSUH112 was allocated.

2.2 Preparation and Extraction Plant Material

The collected sample of *C. integrifolia* stem bark was air dried and pulverized to powder. 2.5 kg of the powdered sample was soaked in methanol for a period of seven days with occasional shaking. The extract was filtered using cotton and Whatmann No.1 filter paper. The marc was again re-soaked in methanol and then filtered off. The combined filtrate was concentrated on a rotavapor at about 45^{o}C to yield a crude methanol extract [9]. The crude extract was reconstituted in methanol: water (1: 9) and successively extracted with n-hexane, ethyl acetate and n-butanol.

2.3 Isolation of Compounds from *C. integrifolia*

The column chromatography purification of n-hexane and ethyl acetate fractions was performed in accordance with the procedure previously reported by Kwaji et al*.* [10] with slight modifications. The n- hexane (15.8 g) and ethyl acetate (4.5 g) extract fractions were dissolved in minimum amounts of methanol and pre-adsorbed on to 20 g silica gel 60 (70-230 mesh) and allowed to dry. Column packing was done using the wet slurry method. Gradient elution was performed and consisted of n-hexane/ethyl acetate and ethyl acetate/methanol at 5% increase in volume of the selected eluting solvents (100:00-100:20 v/v). All eluent fractions were combined based on their thin layer chromatography (TLC) profiles and subsequently concentrated. Fractions with single spot were washed and recrystallized from methanol. Compound CiL1 was isolated as a white substance while CiL2 was yellow in color. Characterization of the isolates were performed using IR, ^{1}H NMR and GC-MS data which were compared with literature.

3. RESULTS AND DISCUSSION

The Table 1 provides a summary of the result of column chromatography purification of the crude extract fractions.

3.1 Characterization of Compound CiL1

The infrared spectrum of CiL1 displayed the following frequencies of vibrations (Table 2).

Table 1. Isolates from ethyl acetate and n-hexane fractions of *C. integrifolia*

SN	Isolate	R_f	Colour	weight	Fraction
1	CiL1	0.81	White	0.4g	n-Hexane
2	CiL2	0.38	Yellow	0.5g	Ethyl acetate

Table 2. FT-IR data of CiL1

SN	Freq. (cm^{-1})	Vibration Type
1	3469	O-H
2	2937	C-H of (CH_3)
4	2869	C-H of CH_2)
5	1634	C=C stretching
6	1466	CH_3 bending
7	1383	CH_2 bending
8	1134	CH_2 bending
9	1049	C-O stretching

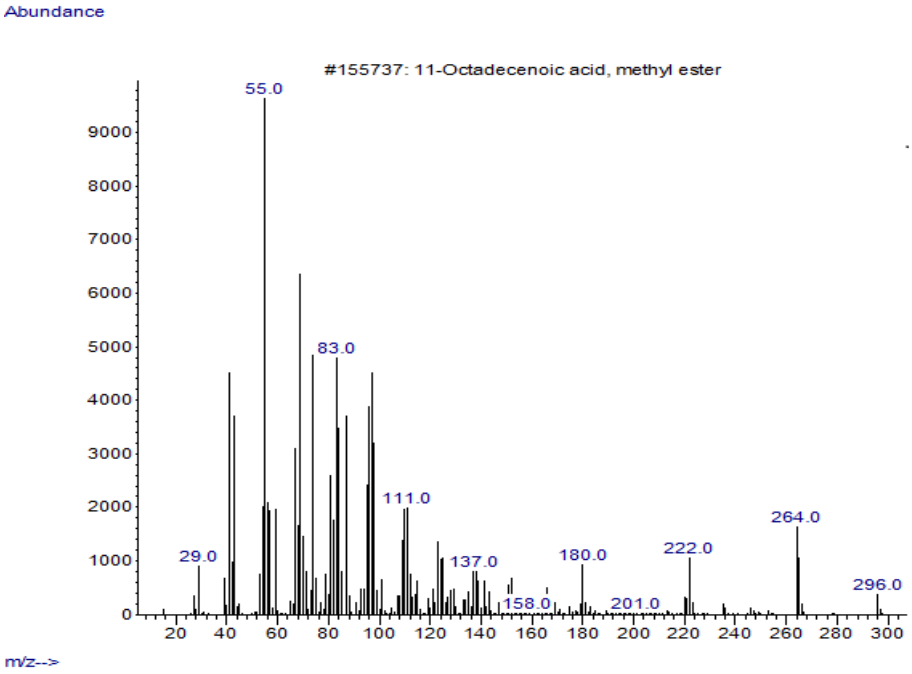

Fig. 1. GC-MS spectrum of CiL1

The peak at 3469 cm^{-1} and 1049 cm^{-1} suggests the presence of bonded hydroxyl (-OH) group of residual methanol solvent while the weak peak at 1634 cm^{-1} indicates C=C olefinic stretching. The stretching and bending vibrations of methyl group was observed as an intense peak at 2937 cm^{-1} and as medium intensity peak at 1466 cm^{-1} while the peaks at 2869 cm^{-1} and 1383 cm^{-1} indicates the presence of methylene groups. The weak peak at 1049 cm_{-1} is due to C-O vibration (Table 2). These absorption frequencies are consistent with literature reports [11,12].

The GC-MS library suggests that the compound CiL1 is 11-octadecenoic acid methyl ester. The molecular formula of 11-octadecenoic acid methyl ester as suggested by GC-MS library is $C_{19}H_{36}O_2$. The molecular ion m/z (M^+) is 296.0 as obtained in the spectrum (Fig. 1). Other daughter ions such as m/z 222 is due to $C_{16}H_{30}$ after removal of $C_3H_6O_2$. The peak at m/z 180 is due to $C_{13}H_{24}$. The removal of CH_3OH from the molecular ion gave rise to the peak at m/z 264. The peak at m/z 111 is due to the fragment $C_7H_{11}O$, while the base peak is due to the fragment C_4H_7O. The observed fragmentation pattern is consistent with literature [11,12].

The ^1H NMR analysis result is in agreement with that of the mass spectrometry. The peak signals at δ_H 5.34 indicates geminal ethylene protons, δ_H 3.52 signifies the presence of methoxy protons, δ_H 2.62 indicates signals for α-methylene group to –C=C-, δ_H 1.84 ppm indicates β-methylene groups to –C-(=O)-O-C, δ_H 1.49 ppm indicates signals of β-protons to –C-C-C= and δ_H 0.69 ppm indicates signal for terminal methyl group of the fatty acid ester. All the above observations are consistent with the molecular structure of 11-Octadecenoic acid methyl ester (Fig. 2).

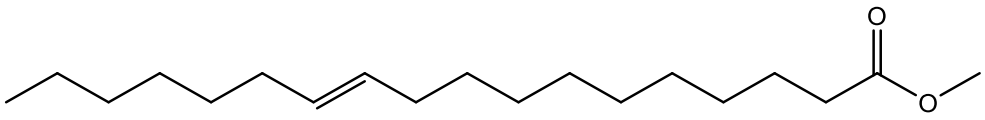

Fig. 2. 11-octadecenoic acid methyl ester

Table 3. FT-IR data of CiL2

S/No	Frequency (cm^{-1})	Type of Vibration
1	3492	OH band
2	1660	C=C stretching
3	1451	CH_3 bending
4	1374	CH_2 bending
5	1292	CH_2 bending
6	1174	C-O-C stretching
7	832	CH bending

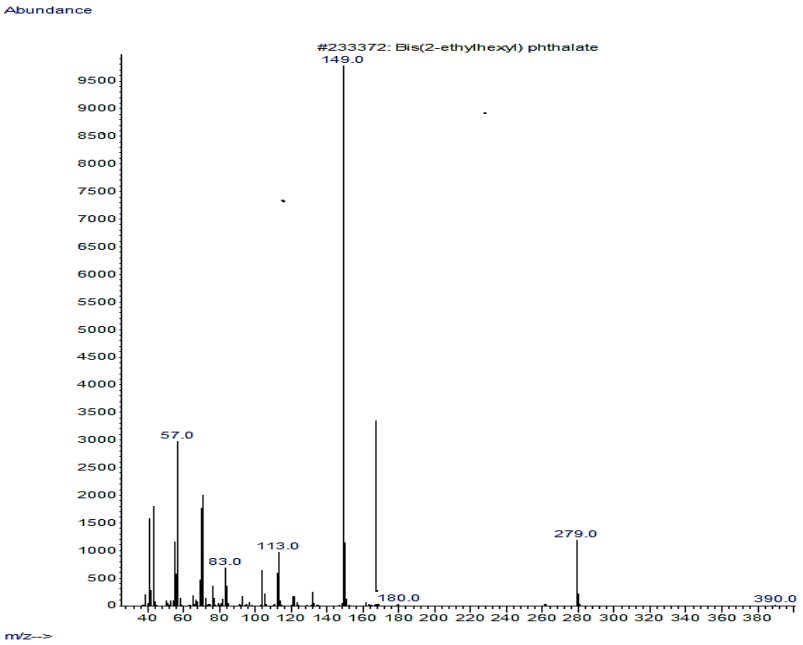

Fig. 3. GC-MS spectrum of CiL2

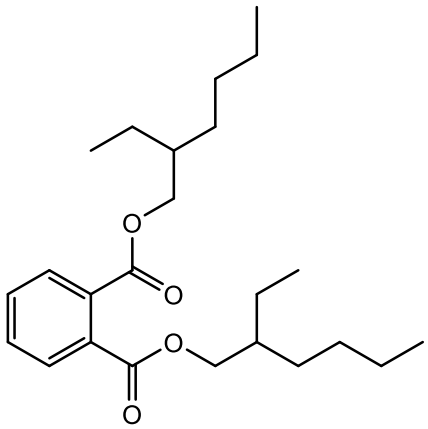

Fig. 4. Bis (2-ethylhexyl) phthalate

3.2 Characterization of Compound CiL2

The compound CiL2 was isolated as a yellow substance and Table 3 shows vibrational peaks from its FT-IR spectrum.

FT-IR result showed a broad peak at 3492 cm^{-1} indicating O-H bond vibrations of hydroxyl group possibly due to residual methanol solvent. The weak vibrations of methyl (CH_3) and methylene (CH_2) groups were observed at 2900 cm^{-1} and 2800 cm^{-1} and as medium intensity peaks at 1451 cm^{-1} and 1384 cm^{-1} respectively. The out of plane C-H vibrations of the unsaturation was observed at 832 cm^{-1}. The corresponding C=C bond vibrations was observed at 1651 cm^{-1} as a weak peak of olefinic bond stretching. The C-O-C bond vibration was observed as a weak intense peak at 1174 cm^{-1}(Table 3). These data summarize the functional groups present in the molecule and suggests that compound (CiL2) is an aromatic ester. These absorption frequencies are consistent with those of bis (2-ethylhexyl) phthalate [13,14,15,16].

The GC-MS library of the compound CiL2 suggested that the compound is bis (2-ethylhexyl) phthalate with 64% abundance and retention time (33.96 minutes). The isolated compound CiL2 parent molecular ion [M^+], m/z, = 390 amu. The base peak m/z = 149 (100). The percentage fragment of other peaks relative to the base peak are m/z - 390(0), 279(12) and 167(37). The parent molecular ion m/z ratio corresponds to the molecular formula $C_{24}H_{38}O_4$ suggesting that the compound is bis (2-ethylhexyl) phthalate (Fig. 3).

The parent molecular ion $[M]^+$ with m/z 390 amu undergoes several cleavages and radical-site rearrangement to give ion fragments with m/z 279, 261, 167, and 149.

The fragment of CiL2 at m/z 279 displayed (Fig. 3) is due to the loss of C_8H_{15} (111 amu). Subsequent fragments at m/z 261 might be due to the loss of a C_8H_{17} (113 amu) and loss of C_8H_{16} (112 amu) yields a fragment ion at m/z 149. The signal at m/z 279 might also be due to loss of C_8H_{16} (112 amu) to give another fragment at m/z 167 followed by loss of H_2O (18 amu) to give a fragment ion at m/z 149 again. The mass fragmentation pattern above is in accordance with previous literature report [17].

Based on ^{1}H-NMR spectrum data of CiL2 ($CDCl_3$; 600Hz,). The two proton signals at δ_H 7.46 ppm and δ_H 7.06 ppm indicates the presence of aromatic ring protons, while the proton signal δ_H 4.86 ppm region indicates the presence of oxygenated methylene protons characteristic of esters. The methylene protons also appear in the region δ_H 3.32-3.21 ppm region and δ_H 1.40-1.30 ppm indicates the presence of aliphatic methyl (CH_3) groups. The methylene protons at the δ_H 4.86 ppm region are more deshielded than those at δ_H 2.78-2.15 ppm as a result of being directly bonded to the electronegative oxygen heteroatom [17]. Consequently, data from IR, ^{1}H NMR, GC-MS and literature strongly suggests that CiL2 is bis (2-ethylhexyl) phthalate.

4. CONCLUSION

The isolation and identification of 11-octadecenoic acid methyl ester and bis (2-ethylhexyl) phthalate in *Celtis integrifolia* Lam crude extract confirms the presence of bioactive compounds. The two compounds are known to possess broad spectrum of antibacterial activity especially against *Escherichia coli* and *Shigella dysenteriae* which are known causative agents of diarrhea. The presence of these compounds in *Celtis integrifolia* is consistent with its traditional medicinal application. Furthermore, the reported cytotoxic nature of these compounds lends credence to the use of the plant in cancer treatment. These substances may act independently or in synergy. The study also showed that the traditional medicinal use of a plant may serve as a guide for the isolation of bioactive compounds.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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