



Isolation and Characterization of Phenolic acid from the Ethanol Leaf Extracts of *Ensete gillettii*

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ABSTRACT

Ensete gillettii (family: *Musaceae*) have been used ethno-medicinally for the treatment of diarrhoea and dysentery. The Crude ethanol leaf extracts was subjected to chromatography which led to the isolation of one carboxylic acid. Characterization and structural elucidation of the carboxylic acid was based on the physical and spectroscopic data (GC-MS, IR, UV, ¹H-NMR, ¹³C-NMR, DEPT - 135°) and literature data. The purification of the crude ethanol leaf extract of *Ensete gillettii* led to the isolation of carboxylic acid, identified as 2-(1,3,5-trihydroxy-7,7,15-trimethylheneicosanyl)benzoic acid.

Keywords: *Ensete gillettii*, isolation, purification, ethanol extracts, characterization

INTRODUCTION

The term medicinal plants include various types of plants used in herbals, and some of these plants can be used for medicinal or therapeutic purposes (Rekha *et al.*, 2013). These medicinal plants are considered as rich sources of active principles which can be used in drug development and synthesis (Okigbo *et al.*, 2008; WHO, 2014). Moreover, these plants play a vital role in the development of human health around the globe (Abbott *et al.*, 2014). Various parts of the plants (leaves, stems, fruits, barks, roots, and flowers) are the essential parts of medicinal plants which provide numerous bioactive compounds useful for therapeutic purposes (Sravanthi-kota and Manthri, 2011). The chemical constituents present in them are part of physiological functions and hence are believed to have better compatibility with human body systems (Partap *et al.*, 2012). These plants are known to play a therapeutic and restorative role in protecting humans from various ailments, hence are considered to have a beneficial role in healthcare need (Barkatullah, 2012). Consequently, there is growing interest in exploiting new drugs from natural and biological active ingredients of plants, which could be used for therapeutic remedies (Nino *et al.*, 2012; Daniels and Malomo, 2014).

Ensete gillettii is a wild banana which has a wide distribution range (West Tropical Africa to Malawi, Benin, Cote d'ivore, Sierra Leon, Nigeria, Togo, Central Republic of Africa, Cameroun and Democratic Republic of Congo) (Scott, 2013). This specie grows in different habitat (grassland and savanna woodlands) but it seems to prefer forest edges on steep slopes which are quite inaccessible areas, making the species less susceptible to human interference (Scott, 2013). *E. gillettii* is a large monocarpic flowering plant and one of the three genera in the banana family, *Musaceae*, with unbranched pseudo-stem of concentric layers of fleshy leaf-petioles arising to 1.5 meters high from a swollen base, not suckering like *Musa* specie, and dying after flowering. It resembles banana plants (Tesfaye *et al.*, 2016), but they have a long, paddle-shaped leaves with crimson midribs. The fruits are similar in appearance to those of banana, but they are dry, seedy plant. The seeds are dark brown, ovoid, and have about 0.4 – 0.5 mm diameter and 0.5 – 0.7 mm long, with a conspicuous white, powdery endosperm. The entire plant dies after fruiting

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and is widely used for household, domestic and personal uses (Afolayan *et al.*, 2014; Tesfaye *et al.*, 2016). *Ensete gillettii* is traditionally used by rural people for the treatment of various ailments. In Nigeria, information from local sources revealed that the root is used to treat various ailments such as dysentery, diarrhoea, stomach ache, digestive disorder, cholera, fever, urinary infection, gonorrhoea, malaria, menstrual pains, typhoid fever, insect bites, skin infections, treatment of wounds and general weakness. The flower, stem and leaves of *E. gillettii* have reportedly been used in the treatment of fever, removal of worms in children, healing wounds treatment of diabetics, diarrhoea and easy delivery among pregnant women (Sethiya, 2016; Tesfaye, 2016). Literature on *Ensete gillettii* however, is scanty, but it has been reported that the ethyl acetate and ethanol fraction of the seeds extracts possess phytochemicals such as alkaloids, flavonoids, phenolic compounds, phlobatannins steroidal compounds and tannins which displayed a promising antibacterial, antioxidant and antifungal activities (Afolayan *et al.*, 2014). In view of this, the paper, presents the isolation and characterization of carboxylic acid from the ethanol extracts of the leaf of *Ensete gillettii*.

METHODOLOGY

Collection and Identification of Plant Materials

Fresh leaves of *Ensete gillettii* were collected from a farmland in Sarkin Pawa, Munya Local Government Area, Niger State, Nigeria in the month of December, 2018. The plant was duly authenticated by a botanist at National Institute of Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria, with a voucher specimen (Voucher no. NIPRD/H/6991) deposited at the Herbarium of NIPRD.

Extraction Procedures

The fresh leaf collected was cut into pieces, air-dried at room temperature. The dried sample was then pulverized. 1 kg of air-dried and pulverized leaf of *Ensete gillettii* was exhaustively extracted with 70 % ethanol by hot maceration continuously at water bath (temperature of 70°C) for several days until the extractant became colorless. The resulting solution was decanted, filtered and then concentrated in *vacuo* and finally dried over water bath to afford a deep brown pasty mass, coded ethanol leaf extracts (L).

Fractionation of Crude Ethanol Leaf Extracts

Vacuum – Liquid Chromatography (VLC) of Crude Ethanol Leaf Extracts

The fresh leaf collected was cut into pieces, air-dried at room temperature. The dried sample was then pulverized. 1 kg of air-dried and pulverized leaf of *Ensete gillettii* was exhaustively extracted with 70 % ethanol by hot maceration continuously at water bath (temperature of 70°C) for several days until the extractant became colorless. The resulting solution was decanted, filtered and then concentrated in *vacuo* and finally dried over water bath to afford a deep brown pasty mass, coded ethanol leaf extracts (L).

Purification of Fraction L1

Pooled column fraction L1 (deep - brown gummy mass, 3 g) was further fractionated using column chromatography (flash chromatography). 90 g of silica gel (60 – 120 mesh) was packed into a column with chloroform (100 %) by slurry method. The dried L1 was mixed with about 5 g of silica gel and allowed to dried after which it was packed into the column followed by addition of chloroform to saturate the column before elution began. Elution proceeded with chloroform (100 %) and continued with increasing polarity of CHCl₃: EtOAc. The eluates were collected in fractions (20 cm³) each and were subjected to TLC using pre-coated plates for chromatographic identification. The identical fractions were pooled to give 1 major column sub-fraction coded L1a.

Re-purification of column sub-fraction L1a

Pooled sub-fraction L1a (1 g, golden brown gummy mass) was resolved by further purification by flash chromatography with 30 g silica gel packed with slurry method using chloroform. Dried L1a was carefully applied to the top of the prepared column as reported above using the same eluting solvents to afford 1 major column sub-fraction coded L1a1 obtained from CHCl₃: EtOAc (9 : 1).

Characterization of the Isolate

IR and UV were recorded both in CHCl₃ using FT-IR (BC2071, Sweden) and UV (210VGP UV-Visible spectrophotometer) respectively, while, GC-MS was recorded using (GCMS Agilent Technologies, 7890A), ¹H-NMR, ¹³C-NMR and DEPT – 135 spectra were taken in

DMSO (Bruker/Topspin, 400 MHz), Visualization of spots Ultraviolet light (254 nm and 366 nm) (Mode UVGL-58, San Gabriel, U.S.A), silica gel 60 F₂₅₄ Aluminium sheets

(Merk, Darmstadt, Germany), silica jel for column chromatography, 60 – 120 mesh (BDH chemicals Ltd Poole, England).

RESULTS AND DISCUSSION

Table 1: Summary of ¹H, ¹³C-NMR and DEPT spectral data of compound L1a1

C/H POSITION	¹ H (δ)	¹ H Multiplicity	¹³ C (δ)	DEPT
1	4.4	Triplet	56.44	(likely CO)
1''	12.0	Weak singlet	171.21	COOH suspected
2	1.9	Triplet	47.33	(-CH ₂ -)
3	3.21	Triplet	59.11	(-CO- likely)
4	1.59	Triplet	40.50	(-CH ₂ -)
5	3.21	Triplet	60.52	(-CO- likely)
6	1.36	Doublet	52.61	(-CH ₂ -)
7	Q _C	-	24.55	Q _C
8	1.21	Triplet	42.32	(-CH ₂ -)
9	1.25	Multiplet	24.63	„
10	1.25	„	30.24	„
11	1.29	„	29.68	„
12	1.25	„	29.93	„
13	1.25	„	27.19	„
14	1.25	„	37.36	„
15	1.25	„	34.22	(-CH-)
16	1.25	„	37.35	(-CH ₂ -)
17	1.25	„	27.22	„
18	1.25	„	29.70	„
19	1.29	„	31.82	„
20	1.31	Sextet	22.70	„
21	0.88	Triplet	14.10	(-CH ₃)
22	0.99	sharp singlet	32.22	„
23	0.99	„	32.21	„
24	0.96	Doublet	21.00	„
1'	Q _C	-	130.83	Q _C
2'	Q _C	-	143.30	„
3'	7.57	doublet of doublet	125.91	(-CH-)
4'	7.72	„	132.83	„
5'	7.59	„	125.86	„
6'	7.93	„	130.83	„
1'''	3.65	Weak singlet	-	-
3'''	3.58	„	-	-
5'''	3.58	„	-	-

*Source: CD/ChemDraw ultra (Product version 12.0); Banfi and Patiny (2008)

Q_C = Quaternary carbon

Compound L1a1 (Figure 1) (golden yellow greasy mass, 10 mg), soluble in chloroform, ethyl acetate and acetone, sparingly soluble in methanol and ethanol, insoluble in water; TLC (CHCl₃ : EtOH, 9 : 1, single spotted, R_f 0.48) (golden yellow in sunlight, golden brown in iodine and pale-brown in UV, bluish-black in FeCl₃. GC-MS revealed its molecular formula

and weight as C₃₁H₅₄O₅ and 506 g mol⁻¹ respectively. IR (ν max, cm⁻¹): 3500 (O-H), 2750 (C-H, aliphatic), 1750 (C=O), 1500 (C=C), 1250 (C-O). UV (λ max, nm): 287, 260 and 210 nm, all the three major bands is typical of π-π* transition of benzene. ¹H-NMR (δ ppm) (Table 1): 4.41 (weak triplet, H-1'), 1.92 (weak triplet, H-2'), 3.21 (multiplet, H-3/H-5), 1.59

(triplet, H-4), 1.36 (doublet, H-6), 1.21 (triplet, H-8), 1.29 (sharp singlet, H-9/H-10, H-13 – 14, H-16 - H-18/ H-11, H-19), 1.20 (sextet, H-20), 0.88 (triplet, H-21), 3.65 (weak singlet, H-1''), 3.58 (weak singlet, H-3''' /H-5'''), 0.99 (sharp singlet, H-22/H-23), 0.96 (singlet, H-24), 7.93 – 7.57 (doublet of doublets, H-6' – H-3'). **¹³C-NMR** (δ ppm): 65.44 (C-1), 47.33 (C-2), 59.11 (C-3), 40.50 (C-4), 60.52 (C-5), 52.61 (C-6), 24.65 (C-7), 42.32 (C-8), 24.63 (C-9), 30.24 (C-10), 29.68 (C-11), 29.93 (C-12), 27.19 (C-13), 37.35 (C-14), 34.22 (C-15), 37.35 (C-16), 27.22 (C-17), 29.70 (C-18), 31.82 (C-19), 22.70 (C-20), 14.10 (C-21), 32.22 (C-22), 32.21 (C-23), 21.00 (C-24), 171.21 (C-1''). 130.83 (C-1'), 143.30 (C-2'), 125.91 (C-3'), 132.83 (C-4'), 125.86 (C-5') and 130.83 (C-6'). **DEPT – 135** (δppm): revealed 4 quaternary carbon (C-7, C-1'', C-1' and C-2'), eight methylene (C-6, C-2, C-14, C-16, C-4, and C-8; C-15; C-11, C-18, C-12, C-10, and C-19; C-13 and C-17; C-7 and C-9, and C-20), and four methyl (C-21 to C-24) groups. **GC-MS** (m/z peaks): 506 [M⁺, C₃₁H₅₄O₅]⁺, 489 [OH]⁺, 461 [CO]⁺, 432 [CHO]⁺, 388[C₂H₄O]⁺, 344 [C₂H₄O]⁺, 288 [C₂H₈]⁺, 218 [C₅H₁₀]⁺, 134 [C₆H₁₂]⁺, 92 [C₃H₆]⁺ and 77 [CH₃]⁺ (base peak). The fragmentation patterns revealed the compound to be a long chain phenolic acid derivative in the form of saturated – branched chain benzoic acids with a characteristic peaks at m/z 134, 92 and 77 (base peak). The molecular ion peak of aromatic acids is large. A moderate benzylic peak is the prominent peaks usually formed by loss of OH (M-17 i.e. M-OH) and COOH (M-45). Further loss of CO (M-28), and CHO (M-29) are typically for carboxylic acids and phenols. This fragment gives the 'aryl' ion (m/z 77) due to McLafferty rearrangement. Generally, a characteristic cluster of ions due to α cleavage and hydrogen migration in mono-alkyl benzene appears at m/z 77 (C₆H₅⁺), 78 (C₆H₆⁺), and 79 (C₆H₇⁺) (Silverstein *et al.*, 1991). IR from the data suggests that the compound is carboxylic acids, while the UV confirmed the compound possesses and sp² hybridized carbon atoms. Proton NMR of the compound indicate the presence of decoupled protons of methine of aryl group (H-6' – H-3'), which was found at δ 133.7 – 125.8. High signal around δ 4.4 and 3.21 (H-1, H-3/H-5) downfield is due the influence of the electron withdrawing oxygen attached to the carbon atom bearing the protons. A weak triplet at δ 1.9 also suggests the electronegative effect of the oxygen atoms

around the protons. Sharp singlet δ 1.25 (H-9 – H-10, H-12 – H-14, H-16 – H-18) confirms the presence of several CH₂ groups resonating in the same chemical environment. Sharp Signal at δ 0.99 and 0.88 resonating upfield suggests the presence of methyl groups; while a weak singlet at δ 12.0 confirm the presence of a carboxylic acid group, typical for carbonyl group. Absence of signal at H-7, also confirms the presence of quaternary carbon. ¹³C-NMR revealed 19 proton decoupled peaks. The weak peak at δ 171.2 is attributable to quaternary carbonyl carbon (C-1''). A carbonyl group usually exhibits anisotropic effect on adjacent atoms due to the influence of the electron withdrawing oxygen. This causes the carbonyl carbon and the protons bonded to neighbouring carbon atoms to resonate at the lowest field (Mohan, 2010). A weak peak at 65.44, 60.52, and 59.11 respectively is as a result of increased downfield effect of methylene carbon attached to an electron withdrawing oxygen atom (-OH) at C-1, C-3 and C-5, respectively (Silverstein *et al.*, 1991). The sharp intense peak between C-14, C-16, C-4 and C-8 ppm suggests the presence of several methylene carbons resonating at the same frequency. A sharp peak also observed between 29.6 to 31.8 ppm (C-11, C-18, C-12, C-10 & C-19) indicates that several methylene carbons are resonating in the same chemical environment. Signal observed as weak peaks at δ 130.8 to 143.3 is an indication of a typical aromatic carbon atoms in which C-1' and C-2' are quaternary carbons (3° carbon), while C-6' to C-3' (methine carbons) are almost in the same chemical environment. The 17-carbon singlet resonances obtained from distortionless enhancement of polarization transfer at pulse 135° (DEPT - 135), it was revealed that 4 peaks were quaternary carbon (disappeared in the spectrum), 8 appeared inverted, while the remaining 9 peaks retained their normal configuration, out of which 3 (aryl-methine carbons), 3 (methane carbons) and 3 (methyl carbons). Generally, DEPT is known to be useful in distinguishing the various types of carbon atoms in a given organic molecules according to the number of hydrogen attached to each carbon (Carey, 2003).

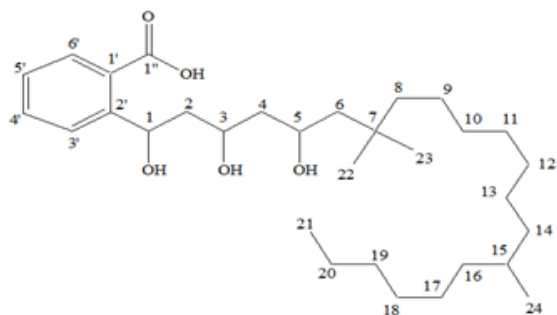


Figure 1: Structure of compound L1a1

CONCLUSION

The purification of the crude ethanol leaf extract of *Ensete gillettii* led to the isolation of carboxylic acid. The compound was characterized using spectroscopic data and identified as 2-(1,3,5-trihydroxy-7,7-15-trimethylheneicosanyl)benzoic acid. Further work will be employed towards isolating and characterizing more bioactive principles from fractions (L2 and L3) in the leaf of the plant.

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