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Isolation and Characterization of a Fatty Acid from the Seed Extracts of *Citrullus lanatus* (Water Melon)

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ABSTRACT

A sample of powdered watermelon seeds sample was macerated with ethanol for two weeks using a maceration method. The mixture was stirred using magnetic stirrer, for 24 hours, in order to extract the powders. It was then decanted, filtered and concentrated on a rotavapor (R110) at 40 $^{\circ}$C in order to obtain the crude ethanol extract (F001). The crude ethanol extract was partitioned into aqueous methanol, chloroform, ethyl acetate and acetone in order to obtain F002, F003, F004 and F005 fractions respectively. The methanol fraction indicated strong activity in the antimicrobial test compared to other fractions. Based on these results, the methanol fraction was subjected to activity-guided chromatographic purification targeting the compound responsible for the observed activity. The methanol fraction (10 g) of the extracted seeds of *Citrullus lanatus* was chromatographed on a silica gel column, using different eluents. This afforded 117 fractions which were combined based on their thin layer chromatography (TLC) pattern. The pooled fraction (-62 – 67-) was further chromatographed in order to obtain the fatty acid. The structure of the compound was characterized – using,- Proton nuclear magnetic resonance (^{1}H NMR), Carbon-13 nuclear magnetic resonance (^{13}C NMR), Distortionless enhancement by-polarization transfer (DEPT), Fourier transform infrared spectroscopy (FTIR) spectroscopic techniques and Mass spectrometry (MS) analysis in order to propose the structure of the compound as a straight chain aliphatic dicarboxylic acid (3-methylnonadec-4,6-diene1,19-dioic acid).

Keywords: Antimicrobial assay, Characterization, Chromatography, Citrullus lanatus

INTRODUCTION

Citrullus lanatus of the family *Cucurbitaceae*, is commonly known as Water Melon (in English) and local named as Kankana (Hausa), Tarmuz (Hindi-) and Puchakaya (Telugu). The ripe fruit is edible and is largely used in making confectionary. Its nutritive values are also known to be useful to the human health. (Rahman *et al.,* 2008, Greenlee 2019 and Renner, 2017).

It has been reported that the seeds of *Citrullus lanatus* show analgesic and anti-inflammatory (Madhavi *et al.*, 2012), anti-ulcerative (Okunrobo, 2012), anti-microbial (Loily *et al.*, 2011), laxative (from the fruit) (Swapnil, 2011), antioxidant (Naresh, 2011) and anti-hyperlipedemic properties (Aruna, 2012).

Citrullus lanatus is a prostrate, climbing annual plant, with several herbaceous, rather firm and stout stems. The leaves are simple, alternate on long petioles, cordate with shallow lobes and on variously serrated margins (FAOSTAT, 2018). These are also, very hairy on the abaxial surface, acute, deep green, and about 7 – 15 cm in diameter (Thomas, 2015). The tendrils are simple and spiral. Male and female flowers grow on the same plant, with yellow petals and green sepals (Amar, 2016). The fruits are globular with shallow grooves, about 14 – 20 cm long. The skin is greenish yellow. The flesh is almost white/light yellow, sweet, delicately flavoured, juicy, a pepo (Deshmukh *et al.*, 2015). The rind of the fruit is prescribed in case of alcoholic poisoning and diabetes (Loiy *et al.*, 2011). The root is purgative and in large doses emetic. The seed is demulcent, diuretic, pectoral and tonic. The Seeds are also vermifuge and have hypotensive action. Fatty oils in the seeds as well as in aqueous and alcoholic extracts paralyze tapeworms and roundworms. In Northern Sudan, it is often used for the treatment of burns, swellings, rheumatism and gout in addition to use as a laxative. Fruits of this plant are eaten as febrifuge when fully ripe or when almost putrid. It is also used in case of energy source, cleanses and purifies kidney and bladder, prevent erectile dysfunction and used to treat hepatomegaly and jaundice (Loiy *et al.*, 2011 and Thirunavukkarasu and Ramanathan, 2010).

This research work was aimed at isolating and characterizing fatty acid from the seed extracts of *Citrullus lanatus.*

MATERIALS AND METHODS

Experimental: All chemical reagents and solvents were purchased from Qualikems (Germany) and Sigma-Aldrich (Germany). Thin-layer chromatography was carried out using precoated

silica gel 60 (F254) that were obtained from MERCK (Germany). Spots on the TLC plates were visualized under UV light (254 nm and 366 nm) and by spraying with 10 % H_2SO_4 in 90 % MeOH followed by heating at 100 ℃ OR Iodine crystal. The melting point of the isolated sample was recorded using a Gallenkamp melting point apparatus. The UV maximum absorption wave length of the extract compound was determined in ethanol using an Agilent CARY 300 UV - visible spectrophotometrer in the visible region of 400 – 800 nm, using a glass cell with 1cm path length. The infrared spectra of the isolated compound were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer using ATR sampling accessory. ^{1}H-NMR and ^{13}C-NMR spectra were recorded using a Bruker Avance III 400MHz spectrometer at room temperature (400MHz for 1H and 100MHz for 13C), using TMS as reference. Measurements were carried out at the University of Sheffield, United Kingdom. Chemical shift values (δ) were reported in parts per million (ppm) relative to TMS and the coupling constants were given in Hz. The solvent used for these measurements was deuterated DMSO. Multiplicities are given as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t) and multiplet (m).

Sample collection and identification

Fresh seeds of *Citrullus lanatus* were collected from Yanlemu market in Kumbotso Local Government Area of Kano State, Nigeria. The seeds were identified and authenticated at Department of Plant Biology, Bayero University, Kano.

Sample preparation and treatment

The seeds were air dried at room temperature for two weeks and crushed into fine powder, using a marble mortar and pestle. A resulting powdered material weighed 700 g, was then stored in a container.

Sample extraction and analysis

The powdered sample (400 g) was macerated with an absolute ethanol (1.5 liters) for two weeks and then, stirred using magnetic stirrer, for 24 hours, in order to part-dissolve the plant material. This was then filtered, and the residue was re-macerated in absolute ethanol (0.75 liters) for a further one week period. This was then decanted, filtered using filter paper and evaporated on a rotavapor (R 110) at 40°C, in order to obtain the crude ethanol extract (F001).

Fractionation of crude ethanol extract (F001)

The crude ethanol extract was partitioned using separatory funnel with solvents of different polarity. The extract was dissolved in 5 % aqueous methanol (100 cm^3) and extracted with chloroform (100 cm^3) to obtained chloroform fraction. The mixture was shaken for some time before being allowed to stand for an hour. Two layers were obtained; aqueous methanol and chloroform layers. The top layer was a methanol fraction and the bottom layer was a chloroform fraction. These were separated and concentrated on a rotavapor (R110) at 40 $^{\circ}$C. The fractions obtained were labeled as F002 and F003 for methanol and chloroform fractions respectively. The Chloroform fraction (F003) was further partitioned with ethyl acetate using (-100 cm^3-) each of chloroform and ethyl acetate. It was then shaken and allowed to stand for an hour. Two layers were obtained and the same procedure, as above, was carried out. The resulting ethyl acetate fraction was labeled F004. The chloroform layer was further partitioned with acetone in a 1:1 ratio. The acetone and chloroform layers were also separated and evaporated one after the other and labeled as F005.

ISOLATION AND PURIFICATION

Packing the column

With the aid of a long glass rod, a clean glass wool was used to block the opening of the column. Silica gel (300 g) was mixed with petroleum ether (900 cm^3) in a beaker and stirred to make slurry. This was poured into the column with the aid of a funnel and continued washing with petroleum ether.

Loading the column

Methanol fraction F002 (5 g) was weighed in a small beaker. The sample was then loaded carefully on top of the silica gel packed inside the column. About 5 g of dried silica gel was added on top in order to serve as a protective layer on top of the sample. Solvents with increasing order of polarity were added to the column. A gradient solvent system of 100 % petroleum ether (1000 cm^3) was initially used to elute the column, followed by petroleum ether-ethyl acetate (200 cm^3) in the order of; 97.5; 2.5, 95.0:5.0, 92.5:7.5, 90.0:10.0, 87.5:12.5, 85.0:15.0, 82.5:17:5, 80.0:220, 77.5:22.5. 75.0:25.0, 72.5:27.5, 70.0:30.0, 67.5:32.5, 65.0:35.0, 62.5:37.5, 60.0:40.0, 57.5:42.5, 55.0.45.0, 52.5:47.5, 50.0:50.0, 40.0:60.0, 30.0:70.0, 20.0:80.0, 10.0:90.0 (%). Subsequently, ethyl acetate 100 % of 200 cm^3 was added to the column, followed by ethyl acetate/methanol (200 cm^3) in this order; 90.0:10.0, 80.0:20.0, 70.0:30.0, 60.0:40.0. 50.0:50.0. The column was finally washed with 100 % methanol (200 cm^3). Different fractions from the column were collected in prepared bottles labeled F002 (1-117), at different time intervals. Each fraction was allowed to evaporate at room temperature, leaving a residue which was subsequently analysed by TLC. Fractions that were similar on the basis of their TLC pattern were pooled (Sharma and Achaya, 1988).

Purification of the pooled fraction (62-67)

Fraction 62-67 (2 g) was subjected to column chromatography using 60 g of 50:200 mesh silica gel and a chloroform/ethyl acetate solvent system. The column was eluted using a gradient solvent system with chloroform and acetone in the following order: 100 % chloroform;- 5:95, 10:90, 15:85, 20:80, 25:75, 30:70, 35:65, 40:60, 45:55, 50:50, 60:40, 70:30, 80:20, 90:10. Subsequently, 100 % ethyl acetate was used and 100 % methanol was used to wash the column.

Thin Layer Chromatography (TLC) analysis

(TLC) analysis was carried out on the crude extracts and the column fractions by using pre-coated aluminium TLC plates (TLC silica gel 60 F_{254}, Merck) (De *et al.*, 2010). The column fractions were spotted on the TLC plates (20 × 20 cm) using capillary tubes and then developed according to the following solvent systems;- petroleum ether (100 %), petroleum ether/ethyl acetate (7:3), (8:2), (6:4), (7.5:2.5) and (4:6). The developed TLC plates were then visualized under a UV lamp at 254 nm until visible spots were circled using a pencil. Iodine vapour was then used in a glass chamber which had a removable glass plate top. The developed TLC plates were placed in the chamber until a dark brown colour was developed on the spots.

Spectroscopic analysis

The isolated compound (-34a-) was subjected to spectroscopic analysis; including ^{1}H-NMR, ^{13}C-NMR, DEPT 135, IR and Mass spectrometry.

RESULTS AND DISCUSSION

Structural elucidation of compound 34a

The compound was isolated as oily liquid substance (12 mg, - Table 1) and subjected for spectral analysis at the University of Sheffield, United Kingdom. The mass spectrum (Figure 1) shows a loss of $–CH_3$ (15 mass unit) in the structure of the compound, while also showing the presence of a fragment with an m/z value of 314. Further loss of – OH (17 mass unit) was observed at the fragment with m/z of 297, and a fragment $–CH_2COOH$ (59 mass unit) corresponds to m/z of 279 from the MS data.

Table 1: Physical characteristics of the isolated compound (-34a-)

Gradient solvent system	R_f value	% yield	Colour	Odour	Texture
Chloroform: ethyl acetate	0.66	12 mg	brown	odorless	Oily

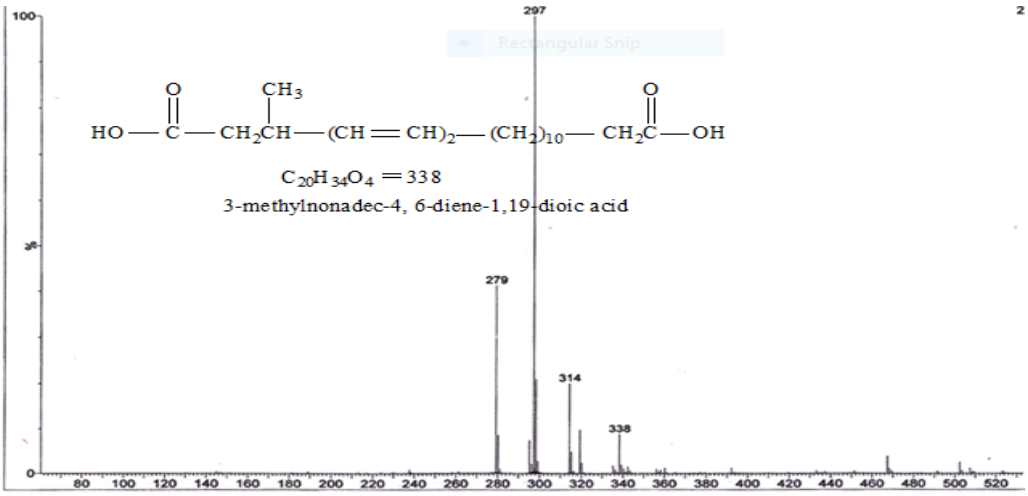


Figure 1: Mass spectrum of 34a

From the FTIR spectrum (Figure 2) and Table 2, two sharp bands at 2921 cm^{-1} and 2852cm^{-1} are observed which represents two different C-H stretching. Another band at 1709.4 cm^{-1} indicates the presence of C=O, associated with carboxylic group. A band at 1261 cm^{-1} is attributed to the stretching of C – O. The IR signals (Figure 2) at 2852, 2921, 1261, 1709cm^{-1} indicated the presence C-H stretching, C-O and C=O. These stretching peaks are typical of carboxylic acids.

Table 2: FTIR numeric data obtained from the analysis of compound (-34a-)

Wave number (cm^{-1})	Functional Group
2852	C-H
2921	C-H
1261	C-O
1709	C=O typical of COOH

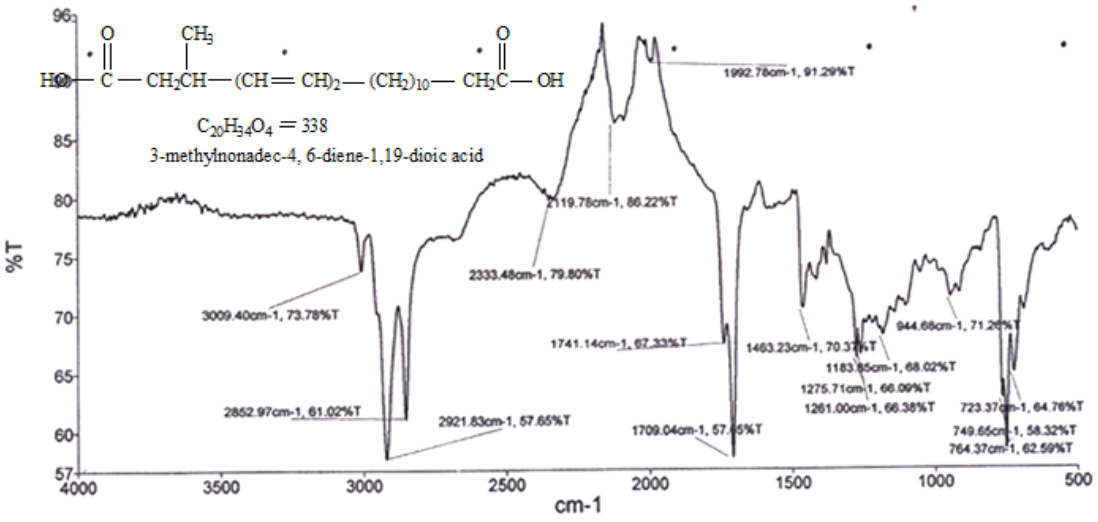


Figure 2: FTIR spectrum of 34a

From the ^{1}H-NMR spectra (Figures 3) and Table 3, a signal at δ 0.85 ppm indicates the presence of methyl protons. While signals at δ 1.55 ppm and δ 1.27 ppm are due to methylene protons. A signal at δ 2.26 ppm with quartet-splitting and that at δ 2.72 ppm, with triplet splitting, are due to methine protons. Signal at δ 3.50 ppm is due to an OH proton. The signal at δ 5.28 ppm is the characteristic of olefinic proton, with multiplet splitting.

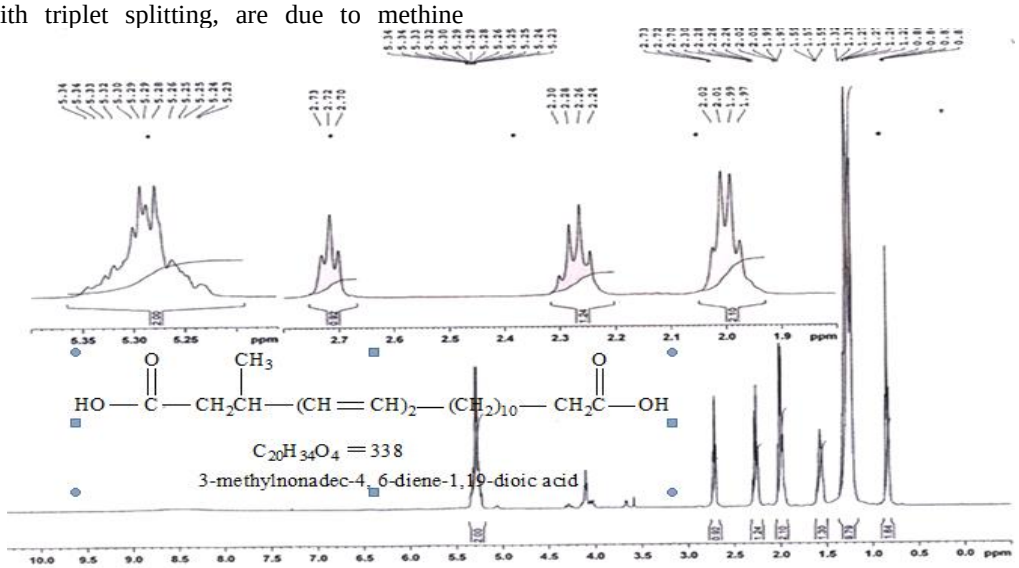
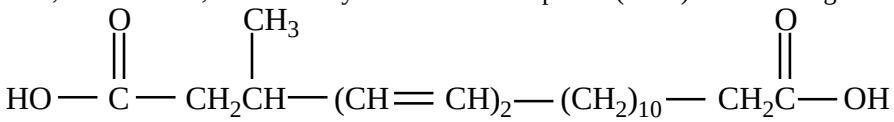


Figure 3: ^{1}H-NMR spectrum of 34a

Table 3: Results from the NMR analysis of compound (-34a-)

^{1}H – NMR of compound 34a			^{13}C -NMR of compound 34a	
Chemical shift value (ppm)	**No of protons**	**Types of protons**	**δ(ppm)**	**C–atom**
0.84	3H	$-CH_3$	22.64	$-CH_3$
1.27	20H	$-CH_2$	24.64	$-CH_2$
1.55	1H	$\diagdown\!\!\diagup CH-$	24.77	$-CH_2$
2.01	4H	$-CH_2$	25.33	$-CH_2$
2.26	1H	–CH=	27.12	$-CH_2$
2.72	1H	–CH=	29.04	$-CH_2$
5.28	2H	–CH=	29.35	$-CH_2$
			29.35	$-CH_2$
			29.36	$-CH_2$
			31.48	$-CH_2$
			31.90	$-CH_2$
			33.95	$-CH_2$
			64.75	$-CH_2$
			67.84	$-CH_2$
			127.83	–CH
			127.98	–CH
			129.74	–CH
			129.80	–CH
			129.91	–CH
			173.66	–COOH
			179.24	–COOH

Consideration of all of the spectral information from the ^{1}H-NMR, ^{13}C-NMR (Figure 4) and DEPT (Figure 5) shows that compound (-34a-) has a total of 20 carbon atoms, out of which, 12 are methylene carbons ($-CH_2$), while 5 are methine carbons (–CH), 2 are carboxylic carbon (–COOH) and only 1 methyl carbon ($-CH_3$). The proposed structure of compound (-34a-) is therefore given as;



$C_{20}H_{34}O_4 = 338$

3-methylnonadec-4, 6-diene-1,19-dioic acid

The compound is a straight chain aliphatic dicarboxylic acid. The structure was confirmed from the mass spectrometry of the compound which gave molecular ion of 338 which correspond with the molecular weight of the compound.

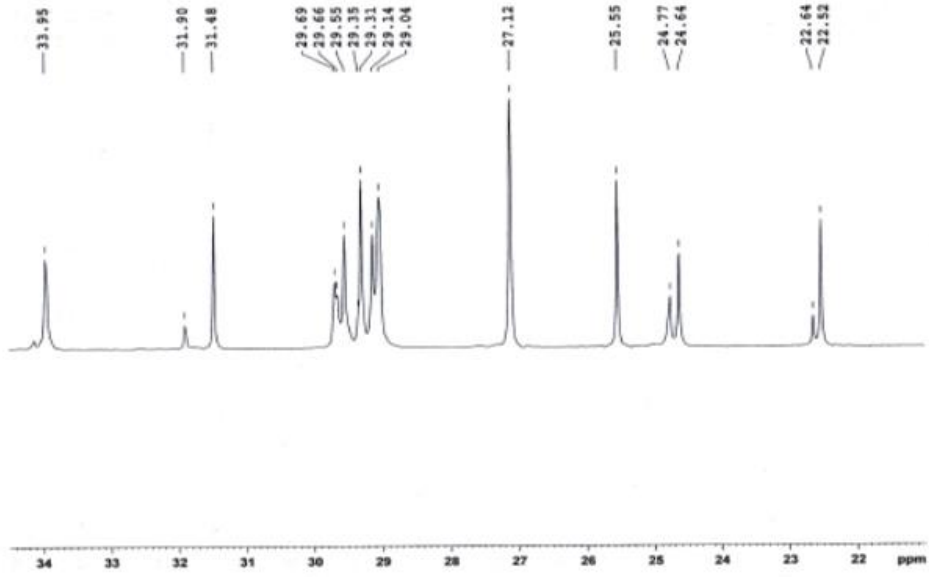


Figure 4: ^{13}C NMR spectrum of 34a

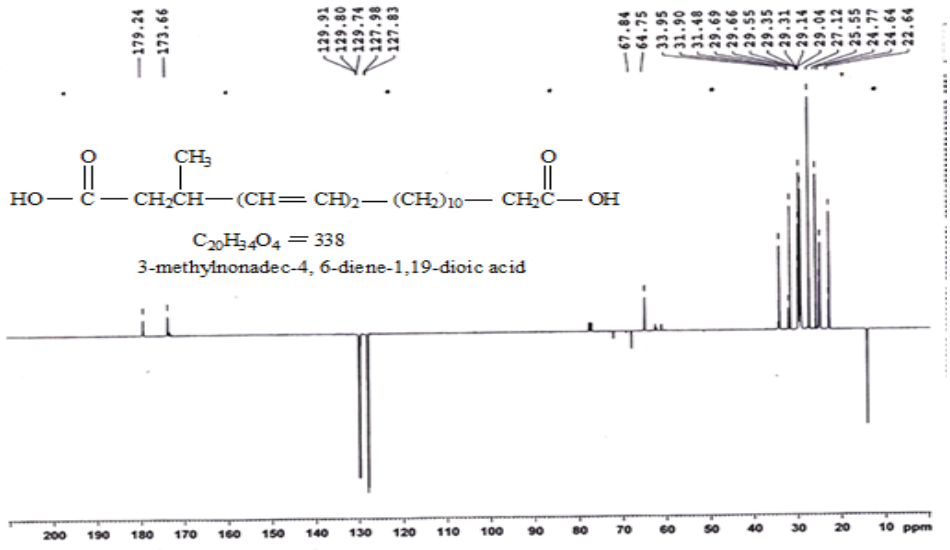


Figure 5: DEPT spectrum of 34a

CONCLUSION

Isolation and characterization of the seeds extracts of *Citrullus lanatus* was successfully carried out. The compound was isolated as odourless oily liquid (12 mg). The structure of the compound was characterized using FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopic techniques and MS spectrometric technique. Analysis and interpretation of the information obtained from all of the spectral analyses- revealed that, the proposed structure of the compound is a straight chain aliphatic dicarboxylic acid.

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