ANTIMICROBIAL ACTIVITY OF N-OCTADECANAL ISOLATED FROM THE SEEDS AND PODS OF *Acacia nilotica* Linn.

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Abstract

The antimicrobial activity of n-Octadecanal isolated from *A. nilotica* was determined using standard methods. The compound was isolated by bioactivity-directed fractionation of ethyl acetate extract of the air dried seeds and pod. The results of the antibacterial screening showed that the ethyl acetate extract of *A. nilotica* exhibited the highest activities against the test microbes with zones of inhibition diameter ranging from 27-32 mm against *Salmonela typhi, Escherichia coli, Streptococcus feacalis, Staphylococcus aureus, Candida krusei* and *Shigella dysentriae*. The structure of the compound was identified from $^{13}C_{nmr}$, $^{1}H_{nmr}$ and GC-MS spectral data. The isolation, structural elucidation, NMR spectral assignment and bioactivities are reported.

Key words; *Acacia nilotica, structural elucidation, antimicrobial activity, bioactivities, spectral assignment.*

Introduction

The World Health Organization (WHO) has listed more than 21,000 plants, which are used for many medicinal purposes around the world (Kathe, 2005). They reported that about 74 % of 119 plant-derived pharmaceutical medicines are used in modern medicine. It also estimates that 4 billion people (80 percent of the world population) presently use herbal medicine for health care. Herbal medicines derived from medicinal plants provides about 75–80 % of the world's population with remedies for health related diseases in both developed and developing countries. Herbs have medicinal property due to the presence of different active principles like alkaloids, volatile essential oils, glycosides, resins, oleoresins, steroids, tannins, terpenes and phenols (Anees, 2010).

It is against this background that *A. nilotica* an extensively used plant in herbal preparation in some parts of Nigeria was investigated. It is commonly called Bagaruwa in Hausa and Booni by the Yorubas in Nigeria and is used in the treatments of intestinal pains, diarrhea, nerve stimulant, cold, congestion, coughs, dysentery, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis (Anees, 2010) The plant was found to be of medicinal importance among traditional medicine practitioners in the tropics, including West Africa. On a wider dimension, the disease causing organism commonly found in the affected sites of the patients is the target of this research. The organisms selected are: *Salmonela typhi, Escherichia coli, Streptococcus feacalis, Staphylococcus aureus, Candida krusei* and *Shigella dysentriae.* The aim of this research is to determine the antimicrobial potentialities of *A. nilotica* and isolate the active compound responsible for the activity.

Methods

Sample collection

The seeds and pods of *A. nilotica* were collected in Nasarawa state. It was identified by

the Herbarium of Biological Sciences, Faculty of Science, and Nigerian Defence Academy and assigned a voucher number of 403.

Extraction

A portion (100 g) of the ground plant parts was separately percolated in 300 cm^3 each of methanol, ethyl acetate, chloroform and petroleum ether each for two weeks. The extracts were separately filtered and evaporated on rotary evaporator at 40^oC. The marcerated was repercolated with the recovered solvents for an additional one week. The extracts were drained, filtered and combined with the previous residue and evaporated on rotary evaporator. Each extract was cooled, weighed and stored in the refrigerator until needed (Garba & Okeniyi, 2012).

Column chromatography of ethyl acetate fraction

Ethyl acetate fraction that showed higher activity in most of the tested microbes was subjected to column chromatography. A portion (20g) of the fraction was dissolved in 80mls of ethyl acetate and mixed with 15g of silica gel. It was evaporated to dryness in a water bath. The dried exract and silica gel were loaded on the column together with 10g of Celite. The column was first eluded with 6:4 ethyl acetate: petroleum ether. This was followed by 8:2 ethyl acetate: petroleum ether, 100% ethyl acetate 1:1 ethyl acetate: methanol and finally
100 % methanol. Each portion collected was evaporated using rotary evaporator (Cannell, 1998). A total of 83 fractions were collected. These fractions were subjected to TLC and similar fractions were pooled together.

Thin Layer Chromatography (TLC)

Fraction 4 was one of the samples that gave a single spot on the TLC plates with an R_f value of 0.65cm using 8:2 petroleum ether: ethyl acetate, R_f of 0.75cm using 7:3 petroleum ether: ethyl acetate and 0.5cm using 9:1 petroleum ether: ethyl acetate.

Antimicrobial activity

The isolates of microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital Zaria from which the zone of inhibition, Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of fraction 4' were determined against the collected isolates of *Salmonela typhi, Escherichia coli, Streptococcus feacalis, Staphylococcus aureus, Candida krusei* and *Shigella dysentriae*

The antimicrobial activities of the pure compound were determined using agar well diffusion methods (Navorro *et. al.,* 1996) and (Okeke *et. al.,* 2001).

Results and discussion

The results of sensitivity test showed that fraction 4 possess different activities against all the test microbes with zone of inhibition diameter of 20, 16, 28, 24, 29 and 22 mm against *Salmonela typhi, Escherichia coli, Streptococcus feacalis, Staphylococcus aureus, Candida krusei* and *Shigella dysentriae* respectively at a concentration of 1000 µg/ml (Table 1). This showed that the fraction could be used for the treatment of various diseases caused by the tested microbes. The results of the MIC for this fraction showed no colour change at $1000\mu g/cm^3$ for all the microbes (Table 2). The lowest concentration where there is no colour change (MIC value), ranges from 4×10^2 - 8×10^2 µg/ cm^3. The results of the MBC showed the MBC value ranges from 10×10^2 - 8×10^2 µg/ cm^3 (Table 3). However, Fraction 4 recorded a broad spectrum of antimicrobial activities against the test microbes. Fraction 4 had a weight of fraction of 0.44g with an R_f value of 0.65cm leading to the isolation of a pure compound whose spectral identity showed that it is n-Octadecanal, with a melting point of 38^0C.

Table 1: Results of the Zone of Inhibition Diameter (mm) of fraction 33 against the test microbes

Test organisms	**1000(µg/cm^3)**	**500(µg/cm^3)**	**250(µg/cm^3)**
Shigella dysentraie	27	20	NI
Escherichia coli	19	10	NI
Staphylococcus aureus	9	4	NI
Salmonella typhi	28	20	15
Streptococcus feacalis	29	24	15
Candida albicans	28	18	12

Key; NI means No Inhibition.

Table 2: The Minimum Inhibition Concentration (MIC) of fraction 33 against the test microbes

	µg/cm^3 (10^2)	**µg/cm^3 (10^2)**	**µg/cm^3 (10^2)**	**µg/cm^3 (10^2)**	**Control**	**µg/cm^3 (10^2)**
Test organisms	**10,8,6,4,2,1,0.5**	**10,8,6,4,2,1,0.5**	**10,8,6,4,2,1,0.5**	**10,8,6,4,2,1,0.5**		**10,8,6,4,2,1,0.5**
Sd	-,-,*,+,+,+,^	-,-,*,+,+,+,^	-,-,*,+,+,+,^	-,*,+,+,+,^,#	Sd	-,*,+,+,+,^,#
Ec	-,-,*,+,+,+,^	-,-,*,+,+,+,^	-,-,*,+,+,+,^	-,*,+,+,+,^,#	Ec	-,*,+,+,+,^,#
Sa	-,-,*,+,+,+,^	-,-,-,*,+,+,+	-,*,+,+,+,+,^	-,*,+,+,+,^,#	Sa	-,*,+,+,+,^,#
St	-,-,*,+,+,+,^	-,-,-,*,+,+,+	-,-,*,+,+,+,^	-,*,+,+,+,^,#	St	-,*,+,+,+,^,#
Sf	-,-,*,+,+,+,^	-,-,*,+,+,+,^	-,-,*,+,+,+,^	-,*,+,+,+,^,#	Sf	-,*,+,+,+,^,#
Ck	-,-,*,+,+,+,^	-,-,*,+,+,+,#	-,-,*,+,+,+,#	-,*,+,+,+,^,#	Ck	-,*,+,+,+,^,#

Key; - means no colour change; * means MIC; + means colour change (light pink); ^ means moderate colour change; # means high colour change.

Control is Ampicloxacillin, St; Means *Salmonela typhi*, Ec means *Escherichia coli*. Sf means *Streptococcus feacalis*, Sa means *Staphylococcus aureus*, Ck means *Candida krusei* and Sd mean

Table 3: The Minimum Bactericidal Concentration (MBC) of fraction 33 against the test microbes

	µg/cm^3 (10^2)	**µg/cm^3 (10^2)**	**µg/cm^3 (10^2)**	**µg/cm^3 (10^2)**	**Control**	**µg/cm^3 (10^2)**
Test organisms	**10,8,6,4,2,1,0.5**	**10,8,6,4,2,1,0.5**	**10,8,6,4,2,1,0.5**	**10,8,6,4,2,1,0.5**		**10,8,6,4,2,1,0.5**
Sd	**-,*,+,#,#^,^**	**-,-,*,+,+,+,^**	***,+,+,#,#,^,^**	**-,*,+,+,+,^,#**	Sd	***,+,+,#,#,^,^**
Ec	***,+,+,#,#,^,^**	**-,-,*,+,+,+,^**	***,+,+,#,#,^,^**	**-,*,+,+,+,^,#**	Ec	***,+,+,#,#,^,^**
Sa	**-,*,+,#,#^,^**	**-,-,-,*,+,+,+**	**-,*,+,+,+,+,^**	**-,*,+,+,+,^,#**	Sa	***,+,+,#,#,^,^**
St	**-,*,+,#,#^,^**	**-,-,-,*,+,+,+**	***,+,+,#,#,^,^**	**-,*,+,+,+,^,#**	St	***,+,+,#,#,^,^**
Sf	***,+,+,#,#,^,^**	**-,-,*,+,+,+,^**	***,+,+,#,#,^,^**	**-,*,+,+,+,^,#**	Sf	***,+,+,#,#,^,^**
Ck	**-,*,+,#,#^,^**	**-,-,*,+,+,+,#**	***,+,+,#,#,^,^**	**-,*,+,+,+,^,#**	Ck	***,+,+,#,#,^,^**

KEY; - means no colony growth, * means MBC/MFC, + means scanty colony growth, # means moderate colony growth, ^ means heavy colony growth. Control is Ampicloxacillin, St; Means *Salmonela typhi*, Ec means *Escherichia coli*, Sf means *Streptococcus feacalis*, Sa means *Staphylococcus aureus*, Ck means *Candida krusei* and Sd means *Shigella dysentriae*

GC-MS Analysis

The GCMS gave the molecular weight of the molecule as 268. The signal at m/z 250 correspond to the loss of water molecule; the signal at m/z 222 correspond to the loss of C_2H_4; the signal at m/z 208 correspond to the loss of CH_2; the signal at m/z 194 correspond to the loss of CH_2; the signal at m/z 180 correspond to the loss of CH_2; the signal at m/z 152 correspond to the loss of CO ; the signal at m/z 124 correspond to the loss of C_2H_4; the signal at m/z 82 correspond to the loss of C_3H_6.

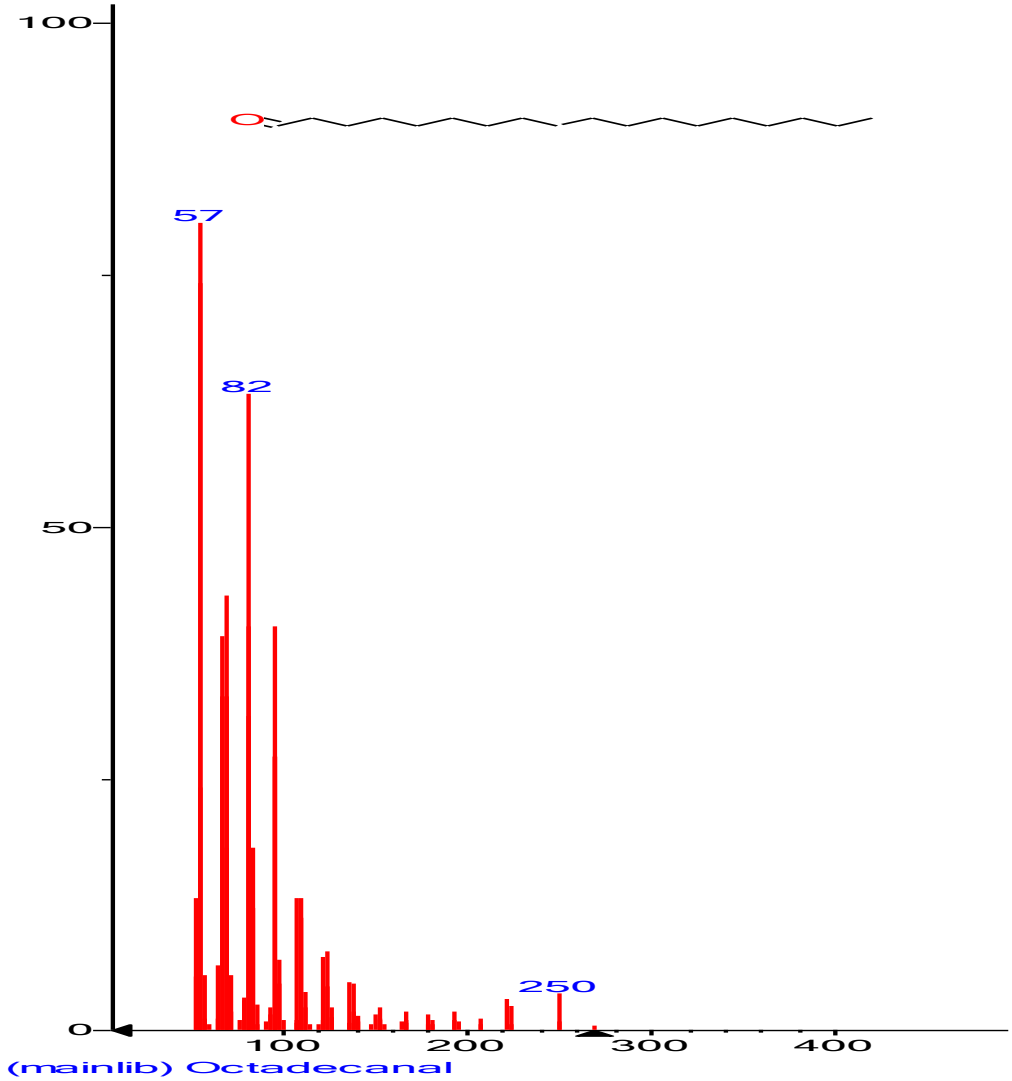

Figure 2; Library search for compound 4

[1]Hnmr Analysis

The [1]Hnmr recorded signals at δ6.8 ppm, which is due to the methine proton (C1); δ3.254, which are methylene protons closer to the –CHO (C2, C3, C4 and C5); δ1.198, which is a methyl proton (C18); δ0.872, which are methylene protons (C6, C7, C8, C9, C10, C11, C12 and C13); δ0.461, are methylene protons closer to the methyl group (C14, C15, C16 and C17).

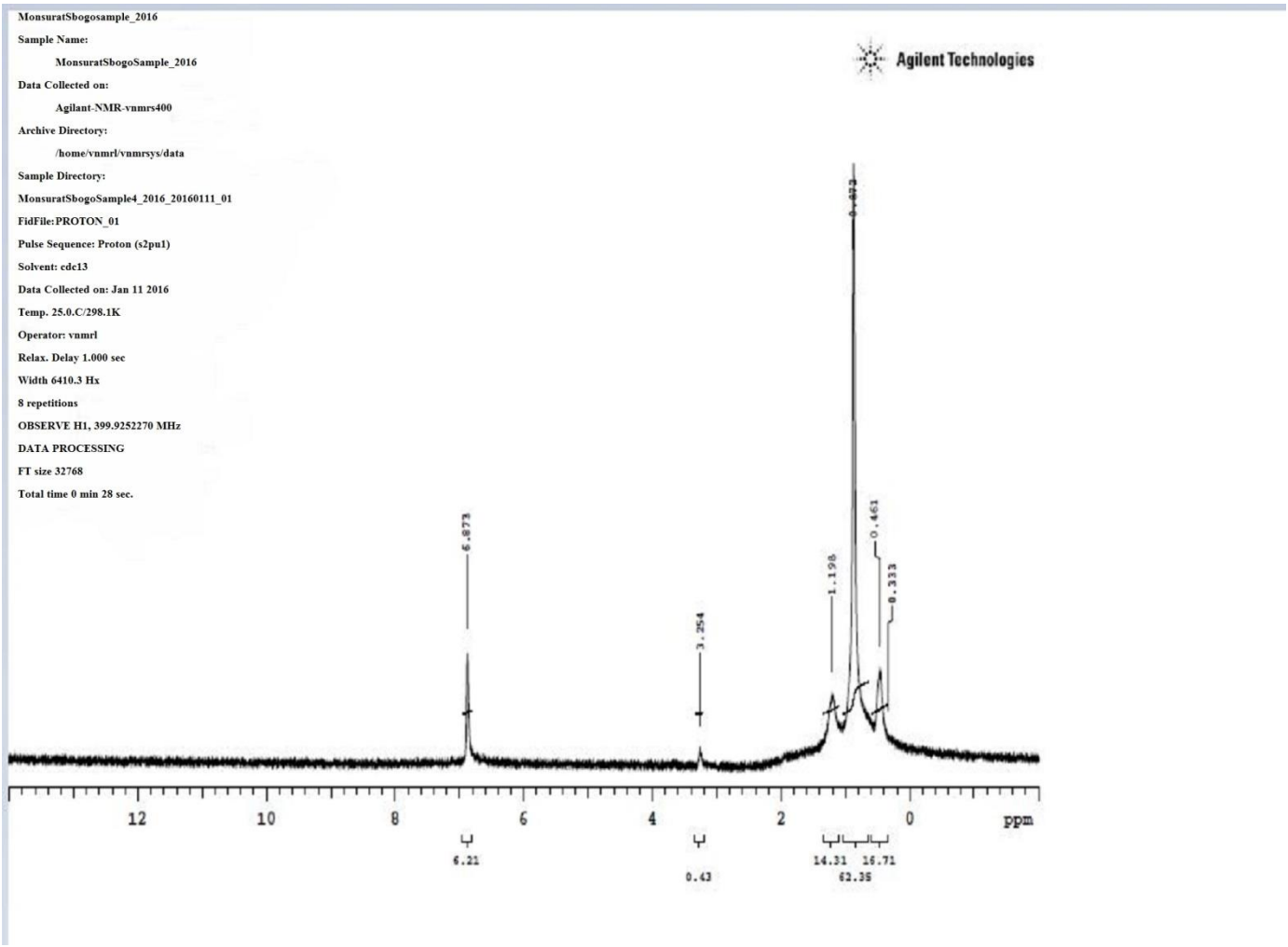

Figure 3: $^{1}H_{nmr}$ of fraction 4

^{13}Cmnr Analysis

The ^{13}Cnmr spectrum had signals recorded at δ173.707, which is due to the –CHO group (C1); δ78.492, δ78.174 and δ77.855, which are due to the methylene carbon directly attached to the –CHO (C2, C3 and C4); δ33.996, δ33.093, δ30.87, δ30.612 and δ30.529 are due to the methylene carbon (C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15 and C16); δ15.272 is due to the methyl carbon (C18); δ23.86 and δ26.917 are due to the methylene carbon attached to the methyl carbon (C16 and C1).

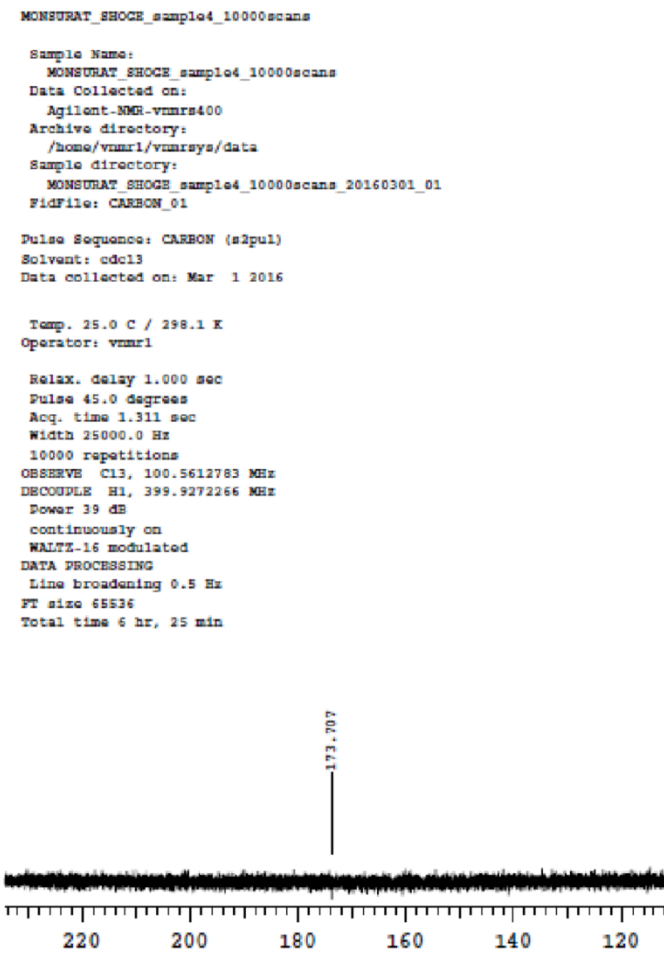

Figure 4: ^{13}Cnmr of fraction 4

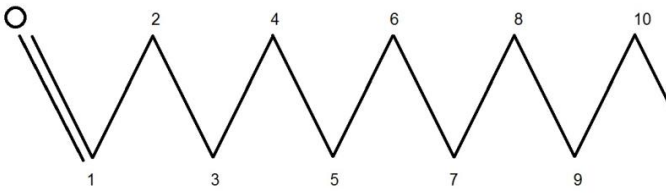

Figure 6: Structure of octadecanal

Conclusion

The ethyl acetate extracts of the seeds and pods of *A. nilotica* Linn was found to have higher activities against the test microbes. Chromatographic separation and thin layer chromatography carried out on it led to the isolation of a compound with a melting point of 38 ^{0}C. This agrees favourably with the melting point of the compound from literature. Structural elucidation using $^{1}H_{nmr}$, $^{13}C_{nmr}$, and GC-MS showed that the compound is n-Octadecanal.

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