



**Isolation and Characterization of Anti-inflammatory Compound from *Ocimum gratissimum* Leaves Extract**

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**Abstract**

*The study was undertaken to identify the possible bioactive compound responsible for the anti-inflammatory agent in Nigerian traditional medicine. Thin layer chromatographic profile of the extract was determined using appropriate solvent system. The compound isolated had a percentage yield of 33.3% and corresponding R<sub>f</sub> value of 0.78 with characteristic aromatic, reddish-brown, oily appearance. Paper and Sudan III tests gave positive test for oil. Subsequent isolation and characterization of the purified compound using column chromatography, alpha-D determination and spectroscopic techniques (UV, IR, NMR and MS) led to the discovery of the proposed compound: methyl-octadeca-9, 12-dienoate also known as methyl linoleate. An increase in the rats hind paw diameters induced by sub-planter injection of a phlogistic agent (formalin 0.1 mL/kg of 2.5 %) was used as a measure of acute inflammation. Ethyl acetate extract was found to have a significant (P <0.01) inhibitory effect on the formalin-induced oedema in rats at the doses of 50, 100, and 200 mg/kg body weight (orally) tested in rats when compared to the normal saline (negative control). The extract also competed significantly (p<0.05) and favourably with standard drug, prednisolone (positive control) at 10 mg/kg body weight. The results confirmed that ethyl acetate extract contains bioactive compound which may be responsible for the anti-inflammatory activity of the plant's leaves and is probably the reasons for the wide-spread use of the plant as anti-inflammatory agent locally and in Nigerian traditional medicine.*

**Keywords:** Anti-inflammatory agent; Bioactive compound; Methyl-linoleate; *Ocimum gratissimum*; phlogistic agents.

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## 1.0 INTRODUCTION

*Ocimum gratissimum* (L.) is an aromatic perennial herb found commonly in tropical Asia especially in India where it is used for aromatic baths in the treatment of rheumatism and paralysis (Madeira *et al.*, 2002). The plant is widely grown in Nigeria, and it is used in the treatment of epilepsy, and high fever, (Okwu and Josiah, 2006). *O. gratissimum* (L.) is commonly known as scent leaf; “Nchuanwa” (Igbo), “Effirin” (Yoruba) “Dodoya” (Hausa), (Orwa *et al.*, 2009; Okoli *et al.*, 2009). The plant is used as food spices (Okwu and Josiah, 2006), and for the treatment of ailments such as malaria, diabetes, respiratory and urinary tract infections, cough, fever, and diarrhea disease (Janssen *et al.*, 1989; Nakamura *et al.*, 1999;

Ngassoum *et al.*, 2003; Offiah and Chikwendu, 1999). Its ability to prevent inflammation of the gum (gingivitis) and mouth (stomatitis) makes *Ocimum gratissimum* leaves extract a good mouth wash to improve oral hygiene (Aguiyi *et al.*, 2000). Bioactivities such as hypoglycemic, anti-inflammatory, antidiarrhoea, antihelminthic and anti-microbial of the plant extracts confirmed its broad spectrum of applications in traditional medicine, (Dubey *et al.*, 2000; Okoli *et al.*, 2009; Pessoa *et al.*, 2002). The research objectives are to isolate bioactive compound from ethyl acetate fraction, and to establish the structure of the compound isolated through spectroscopic means.

## 2.0 MATERIALS AND METHODS

### 2.1 Collection of Plant Materials

Fresh leaves and stems of *O. gratissimum* were collected from the Usmanu Danfodiyo University Teaching Hospital Staff Quarters in August 2014, in a garden, where they were grown as economic plants.

### 2.2 Identification of Plant Materials

The plant was identified in August 2014 by a Taxonomist at the herbarium of the Department of Pharmacognosy and Ethno-pharmacy, Usmanu Danfodiyo University, Sokoto. A voucher specimen with identification number: Pcg/udus/0001 was deposited at the herbarium for reference purpose.

### 2.3 Location of Study

This research was carried out partly at the Department of Pharmacognosy and Ethno-pharmacy, Faculty of Pharmaceutical Sciences, and the rest at the Department of Pure and Applied Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.

### 2.4 Chemicals

General laboratory chemicals, drugs and reagents of analytical grade were used.

### 2.5 Chromatographic Studies

**2.5a Thin Layer Chromatography (TLC) procedure:** Thin layer chromatography was conducted on the plant extract in order to

separate the components as described by Arnason *et al.* (1995).

**2.5b Column Chromatography (CC) of Ethyl acetate Extract:** Silica gel (120.0g; mesh-sizes 60-200), was wet packed in a glass column (60 cm x 2.5 cm). The column was wet packed in 100% hexane. The extract (3.0 g) in a fine powder form was loaded onto the column packed with silica-gel and allowed to stabilize for two hours before elution commenced. The column was eluted in gradient profile. Nine stages of polarity were used in the fractionation and purification process. Purification was attained at 80:20 hexane/EtOAc solvent system. Pure fractions with similar TLC profiles were collected together and thereafter concentrated, crystallized and labeled as ‘Isolate A’.

### 2.6 Animal Management

Male adult Wistar rats weighing between 150-250 g were used for the experiments. The animals were obtained from animal house of Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto, they were randomly divided into 5 groups of 5 animals per group, maintained under standards environmental conditions of temperature, illumination of light and dark cycle. Ethical clearance for handling the animals and the procedures used in the study was obtained from the institutional animal ethical committee.

## 2.7 Spectral analyses of compound A

### 2.7a Ultraviolet/Visible Spectroscopic

**Analysis:** The compound isolated was analysed using UV/vis spectrophotometer over the wavelength range of 200-800 nm and absorbance units of 0.00-2.00.

**2.7b Infrared Spectroscopy (IR):** The IR analysis was carried out on the isolated compounds using the Perkin Elmer Spectrum RX FT-IR System.

### 2.7c Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopic experiment carried out includes:  $^1\text{H}$ ,  $^{13}\text{C}$  and DEPT-135). NMR spectra were obtained at 300K on Top spin 400 MHz NMR spectrometers (Bruker Germany). Chemical shifts ( $\delta$  values) in (ppm), coupling constant (J- values) in Hz and multiplicity recorded.

### 2.7d Gas Chromatography/Mass spectrometric Analysis

The compound isolated was analyzed using GC-MS Agilent Technology. The molecular ion  $\{M\}^+$  and other fragments ions were obtained by electron impact mass spectroscopy (EIMS)

## 2.8 Formalin-induced Paw Oedema in Rats

An increase in the rat hind paw diameter induced by sub-planter injection of a phlogistic agent was used as the measure of acute inflammation (Winter *et al.*, 1963). The

phlogistic agent employed in this study was formalin 0.1 mL/kg of 2.5 %. The rats were randomly divided into 5 groups of 5 animals per group. Thirty minutes before the injection of formalin, the groups were treated orally as follows. group 1: 0.9 % (w/v) normal saline 10 cm<sup>3</sup>/kg, (negative control), group 2: 1 mg/kg prednisolone (as positive control), groups 3, 4 and 5 received ethyl acetate leaves extract of *O. gratissimum* at the doses of 50, 100 and 200 mg extract mg/kg respectively. Paw thickness was measured hourly after formalin injection. Paw diameter (mm) was measured at 1, 2, 3, 4 and 5 hours later using digital caliper, and percentage inhibitions determined.

$$\text{Inhibition (\%)} = \frac{\text{MPD (c)} - \text{MPD (t)}}{\text{MPD (c)}} \times 100$$

MPD (c)

MPD = mean paw diameter, c = control, t = treated.

## 2.9 Data Analysis

All data in the experiment was expressed as mean  $\pm$  standard error of the mean (S.E.M), while statistical significance between groups was done using student's T-test. Differences in mean were considered to be significant when  $p < 0.05$  and  $p < 0.01$  (Duncan *et al.*, 1977).

## 3.0 RESULTS

### 3.1 Thin Layer Chromatography

Preliminary TLC profile of ethyl acetate (solvent system: ethyl acetate/hexane 80:20) showed distinct resolution of coloured spots with varying  $R_f$  values (0.34, 0.6, 0.7 and 0.78). The TLC profile was considered for column chromatography.

### 3.2 Isolation and Purification of the Extract

Nine stages of polarity were used in the fractionation and purification process and the respective characteristics of various eluates from the column grouped according to their

chemically similar profiles. Purification was attained at 80:20 hexane/EtOAc. The compound isolated (Compound A), had a percentage yield of 33.3% and corresponding  $R_f$  value of 0.78 with characteristic aromatic, reddish-brown, oily appearance. Paper test and Sudan III tests gave positive test for the oil.

### 3.3 Ultra Violet –Visible Spectroscopy of Compound A

The compound showed absorption over the wavelength of 245 nm as indicated in Figure 1. The absorbance value of 1.814 can be attributed to the presence of chromogenic compound.

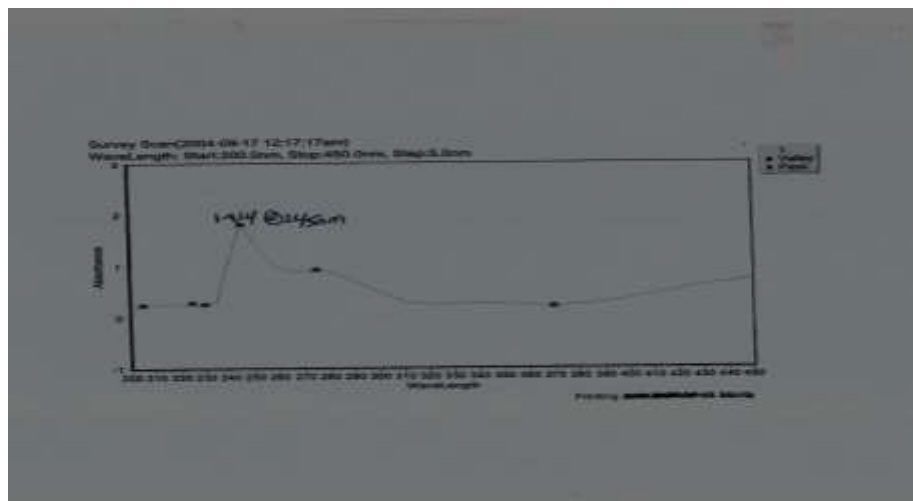


Figure 1: UV-VIS Spectrum of Compound A.

### 3.4 Infra-Red (IR) Spectroscopy of Compound A

Infrared (KBr) spectrum of the compound showed characteristic peaks at  $\nu_{\max}$  ( $\nu$ ,  $\text{cm}^{-1}$ ) 1733.76  $\text{cm}^{-1}$  (C=O stretching band of methyl esters), 1171  $\text{cm}^{-1}$ , 1177.36  $\text{cm}^{-1}$ , and 1246.70  $\text{cm}^{-1}$  (C-O stretching), 1651  $\text{cm}^{-1}$  (C=C

stretching), 2852.89 and 2923.74  $\text{cm}^{-1}$  (C-H stretching) while the absorbance at 3000  $\text{cm}^{-1}$  indicates the =C-H stretching frequency and the observation of an absorption peak at 735  $\text{cm}^{-1}$  suggested the  $\text{CH}_2$  rocking and C-O stretching bands at 1171, 1177.36, and 1246.70  $\text{cm}^{-1}$ .

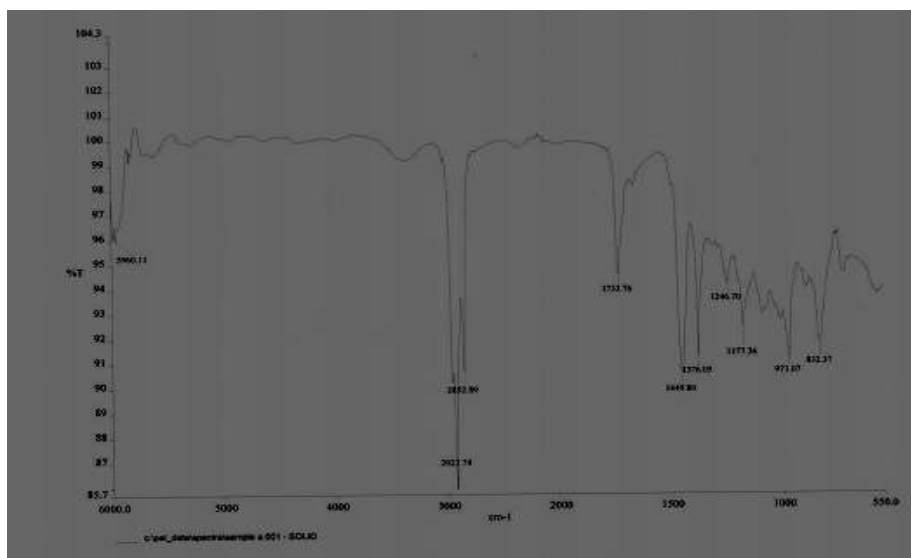


Figure 2: IR Spectrum of Compound A (Frequency/ $\text{vcm}^{-1}$ ).

### 3.5 Proton Nuclear Magnetic Resonance Spectroscopy (<sup>1</sup>HNMR) of Compound A

The <sup>1</sup>HNMR spectrum of the compound in Figure 2 showed the following signals: doublet at  $\delta$  5.31-5.36 ppm, a singlet at  $\delta$  3.65 ppm, bis-allylic proton signals appear at around  $\delta$  2.8 ppm;  $\alpha$ -methylene protons to double bond (-CH<sub>2</sub>-

C=C-) appear as a singlet at 2.05 ppm. The  $\beta$  methylene protons to ester (CH<sub>2</sub>-C-CO<sub>2</sub> Me) also appear as a multiplet at 1.59-1.63 ppm. The singlet signal at 1.24 and 1.3 are expected for the protons of backbone methylenes of long fatty acid and chain. The terminal methyl proton (C-CH<sub>3</sub>) at  $\delta$  0.86-0.96 ppm appears as a multiplet.

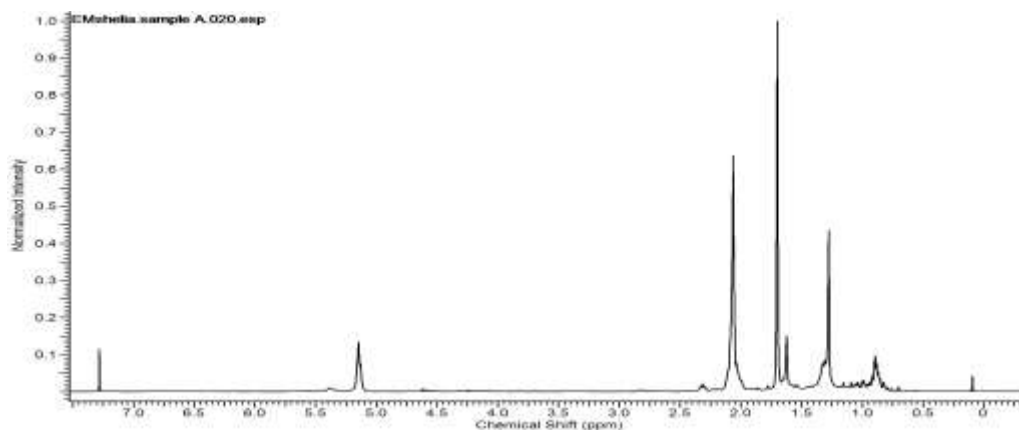


Figure 3: <sup>1</sup>HNMR Spectrum of Compound A in CDCl<sub>3</sub>, 400 MHz.

### 3.6 <sup>13</sup>CNMR Spectrum of Compound A

Several signals were observed in the spectrum as shown in Figure 4. A typical signal of the carbonyl carbon of the ester molecules is expected at  $\delta$  174 ppm and the olefinic carbons appear at around  $\delta$  126 and 137 ppm. The

expected signal at  $\delta$  51.2 ppm is due the methoxyl carbon esters. The methyl and methylene carbons of fatty acids appear in the range from  $\delta$  13.92 and 33.93 ppm.

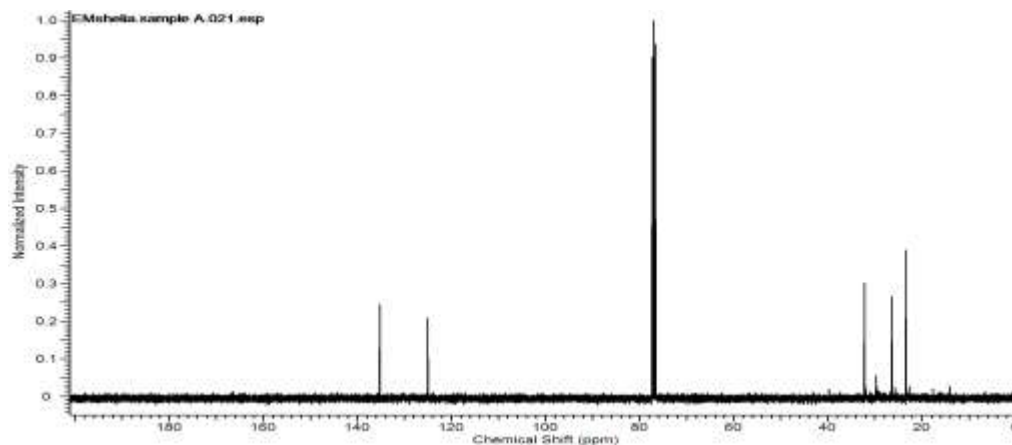


Figure 4: <sup>13</sup>CNMR Spectrum of Compound A in CDCl<sub>3</sub>, 400 MHz.

### 3.7 Distortionless Enhancement by Polarisation Transfer (DEPT) Spectrum of Compound A

The experiment was conducted to distinguish the different types of carbon present in the compound. The DEPT-135 of compound A showed 10 positive signals due to methine (CH)

and methyl (CH<sub>3</sub>) at  $\delta_c$  (ppm): 22.1, 26.0, 24.0, 28.0, 30.0, 39.0, 44.0, 46.0, 126.0 and 140.0. Eight negative signals due to methylene (CH<sub>2</sub>) were seen at  $\delta_c$  (ppm): 23.0, 26.0, 28.0, 32.0, 34.0, 39.0, 125.0 and 128.0. The number and type of carbon present is consistent with the structure of methyl -9, 12-octadecadienoate.

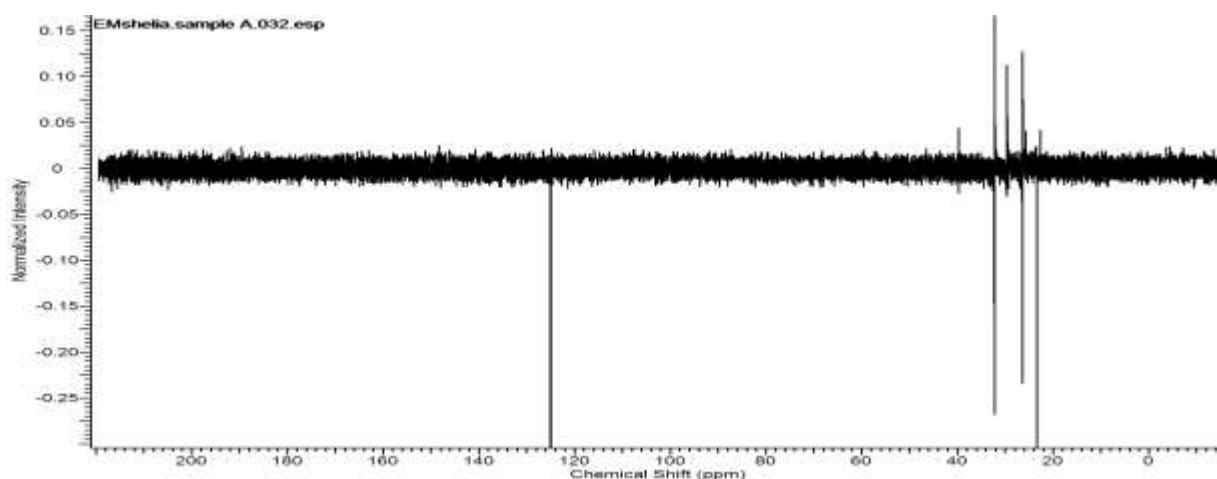


Figure 5: DEPT-135 NMR Spectrum of Compound A in CDCl<sub>3</sub>, 400 MHz.

### 3.8 Mass Spectrometry of Compound

The molecular ion (M)<sup>+</sup> and other fragment ions were obtained by electron impact mass spectroscopy (EIMS). The mass spectrum

showed the molecular ion at M/Z 298 with characteristic peaks of other fragment ions at M/Z 269, 255, 199, 185, 143, 129, 87, and 74.

Hit#:1 Entry:370822 Library:Wiley9.lib

SI:88 Formula:C19H38O2 CAS:112-61-8 MolWeight:298 RetIndex:0

CompName:Octadecanoic acid, methyl ester (CAS) SS Methyl stearate SS Methyl octadecanoate SS Methyl n-octadecanoate SS Stearic acid methyl ester SS

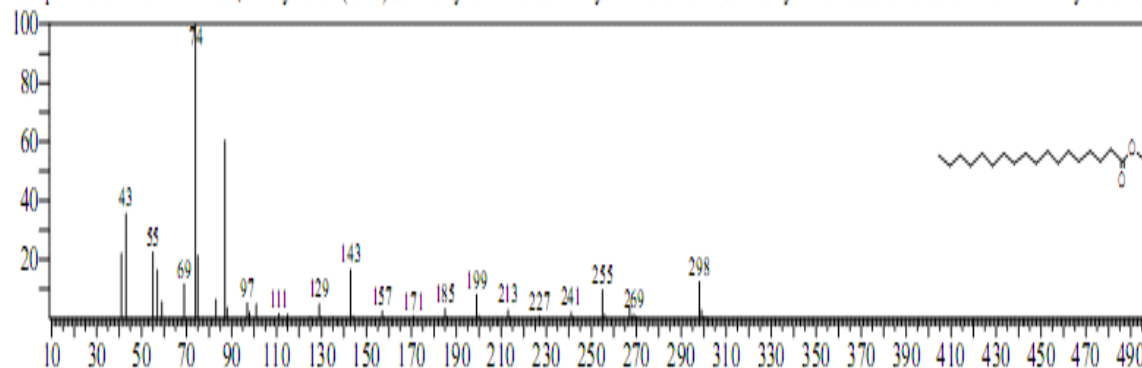


Figure 6: Mass Spectra of Isolated Compound.

### 3.9 Optical Activity of Compound A

Optical activity of the isolated compound was determined using Alpha-D Spectrophotometer. The specific rotation of the compound was  $4.5^{\circ}$ .

### 3.10 Anti-Inflammatory Study of Ethyl acetate Extract

Anti-inflammatory study was conducted on 25 healthy and mature rats. Increases in the rat hind paw diameter induced by sub-planter injection of a phlogistic agent (formalin 0.1cm<sup>3</sup>/kg of 2.5

% w/v) were used as the measure of acute inflammation. The extract was found to have a significant ( $P < 0.01$ ) inhibitory effect on the formalin-induced oedema in rats at the doses of 50, 100, and 200mg/kg body weight (orally) tested in rats when compared to the normal saline (negative control) as shown in Table 1 below. The extract also competed significantly ( $p < 0.05$ ) and favourably with standard drug.

Table1: % inhibition of Normal saline (0.9 w/v), Ethyl acetate, and Prednisolone on Formalin-induced Paw Oedema in Rats

Group	Dose mg/kg	Percentage(%) inhibition				
		1hr	2hr	3hr	4hr	5hr
Normal saline n=5	10 mL/kg					
Ethyl acetate n=5	50	49.1	*56.5	*58.5	*61.5	*65.5
Ethyl acetate n=5	100	*68.7	*69.5	*71.4	*73.0	*74.4
Ethyl acetate n=5	200	**75.7	**76.2	**76.8	**77.8	**77.3
Prednisolone n=5	1.0	**76.7	**78.4	**77.8	**76.8	**76.4

An appreciable significant hourly inhibition of formalin when compared to standard drug was observed: \* $P < 0.05$ ; \*\* $P < 0.01$ . Values are expressed as  $\pm$  S.E.M (Standard Error of the Mean) of five rats using Student's T-test

### 3.11 DISCUSSION

A signal at M/Z 74 was produced on the spectrum of the GC-MS (Figure 6) as a result of McLafferty rearrangement that occurred in the molecule which gives useful information about the structural arrangement of the compound; it is typical of fatty acid methyl ester and is easily earmarked from the spectrum by observing the base peak (M/Z 74 (100%), formed by the  $\beta$ -

cleavage in the McLafferty rearrangement mechanism (Burchill *et. al*, 2007). The molecular ion peak 298 represents the intact molecule. However, the mass spectrum did not provide information about the pi ( $\pi$ ) bond system in the isolated compound. Detailed information about the presence of double bonds between C<sub>9</sub> - C<sub>10</sub>, and C<sub>12</sub> - C<sub>13</sub> carbon atoms of

the compound was provided by the IR and NMR spectra (Figure 2, 3, 4 and 5). The  $^1\text{H}$ NMR spectrum of the compound as shown in Figure 2 showed a characteristic doublet at  $\delta\text{H}$  5.31-5.36 ppm represents the olefinic protons (-CH=CH-) (Pravia *et. al.*, 2001). The bis-allylic proton signals of polyunsaturated fatty acid appear at around  $\delta\text{H}$  2.8 ppm while  $\alpha$ -methylene protons to double bond (-CH<sub>2</sub>-C=C-) appear as a singlet at 2.05 ppm (Pravia *et. al.*, 2001). All the information suggested the presence of  $\pi$  electrons in the compound (Wong *et. al.*, 2001). The  $\beta$  methylene protons to ester (CH<sub>2</sub>-C-CO<sub>2</sub>Me) also appear as a multiplet at  $\delta\text{H}$  1.59-1.63 ppm and a singlet at  $\delta\text{H}$  3.65 ppm suggested the presence of methoxyl protons of the ester functionality (Pravia *et. al.*, 2001). The singlet signal at  $\delta\text{H}$  1.24 and 1.3 are expected for the protons of backbone methylenes of long fatty acid and chain (Pravia *et. al.*, 2001). The terminal methyl proton (C-CH<sub>3</sub>) at  $\delta\text{H}$  0.86-0.96 ppm appears as a multiplet confirmed the presence of the second methyl signal in the compounds (Pravia *et. al.*, 2001). These data are supported by  $^{13}\text{C}$ -NMR spectrum and DEPT-NMR. The spectrum of  $^{13}\text{C}$ -NMR showed expected typical signal of the carbonyl carbon of the ester molecules at  $\delta\text{C}$  174 ppm and the olefinic carbons appear at around 126 and 137 ppm confirming the presence of 2  $\pi$  bond systems in the compound (Pravia *et. al.*, 2001) thereby complementing their absence in the mass spectrum. The demonstrated signal at  $\delta\text{C}$  51.2 ppm confirmed the presence of functionality of methoxyl carbon esters (Pravia *et. al.*, 2001). The DEPT-135 positive and negative signals seen on the spectrum are diagnostic and number and types of carbon present is consistent with the

structure of methyl -octadeca-9, 12 dienoate. Infrared (KBr) spectrum of the compound showed characteristic peaks at  $\nu_{\text{max}}$  ( $\nu$ ,  $\text{cm}^{-1}$ ) 1733.76  $\text{cm}^{-1}$  (C=O stretching band of methyl esters), 1171  $\text{cm}^{-1}$ , 1177.36 $\text{cm}^{-1}$ , and 1246.70  $\text{cm}^{-1}$  (C-O stretching), 1651  $\text{cm}^{-1}$  (C=C stretching), 2852.89 and 2923.74  $\text{cm}^{-1}$  (C-H stretching) while the absorbance at 3000  $\text{cm}^{-1}$  indicates the =C-H stretching frequency and the observation of an absorption peak at 735  $\text{cm}^{-1}$  suggested the CH<sub>2</sub> rocking (Pravia *et. al.*, 2001). The ultraviolet spectrum absorbance value at 1.814 is attributed to the presence of  $\pi$  bond electrons (Pravia *et. al.*, 2001). The information derived from the MS, NMR, IR, UV and alpha-D is consistent with the structure of methyl octadeca-9, 12-dienoate (methyl linoleate), a fatty acid methyl ester of linoleic acid. Ethyl acetate extract of *O. gratissimum* leaves produced very significant anti-inflammatory effects at all doses tested (Table 1). An appreciable significant hourly inhibition of formalin-induced edema in rats when compared to standard drug was observed: \*P < 0.05, and \*\*P < 0.01, when compared to negative control (Table 1). Ethyl acetate extracts at all the doses, demonstrated increase inhibition at dose-dependent and steady rate (Table 1). The inhibitory property of ethyl acetate extract was used as a measure of its anti-inflammatory ability (Winter *et al.*, 1962).

### 3.12 Proposed Structure of Compound A

From the MS, NMR, IR, UV and Alpha-D data which compared well with available literature, the suggested structure of Compound A is Methyl octadeca-9, 12,-dienoate.

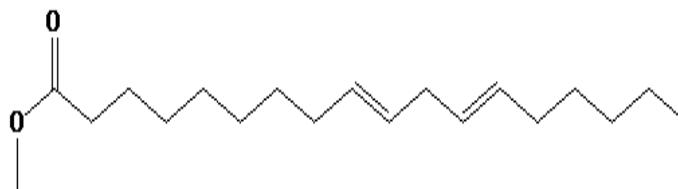


Figure 7: Proposed Structure of Compound A.

## 4.0 CONCLUSION AND RECOMMENDATIONS

### 4.1 CONCLUSION

In the course of the research, *O. gratissimum* leaves have been evaluated and the study showed that it exhibited anti-inflammatory activity. Anti-inflammatory bioactive compound was isolated from ethyl acetate extract of the plant. Elucidation and characterization to establish the structure of the isolated compound was carried out through series of column chromatography and spectroscopy techniques.

### 4.2 RECOMMENDATIONS

It is evident from this work that ethyl acetate, a moderately polar solvent could successfully be employed to isolate a bioactive compound from *O. gratissimum* plant leaves. Further efforts should be made to employ the use of non-polar and more polar solvents for comparative studies.

## 5.0 REFERENCES

- Aguiyi, J. C., Obi, C. I., Gang, S.S. and Igweh, A.C. (2000). Hypoglycaemic activity of *Ocimum gratissimum* in rats. *Fitoterapia*, 71: 444-446.
- Arnason, J.T., Mata, R., and Romeo, J.T. (1995). *Phytochemistry of medicinal plant: Recent advances in Phytochemistry Vol. 29*; Plenum Press, New York; 364 pp.
- Burchill, P. Herod, A.A. and Marsh, K.M. (2007). Gas chromatography in water analysis: Selective detection methods. *Water res*; 17 (12):1905-1916.
- Dubey, N. K., Tiwari, T. N., Mandin, D.; Andriamboavonjy, H. and Chaumont, J. P. (2000). Antifungal properties of *Ocimum gratissimum* essential oil (ethyl cinnamate chemotype). *Fitoterapia*; 71, 567-569.
- Duncan, R. C., Knapp, R.G. and Miller, M. C. (1977). Test of hypotheses in population means: Introductory Biostatistics for Health Sciences, John Wiley and Son, Inc. N. Y. pp. 71 – 76.
- Janssen, A. M., Scheffer, J. J. C., Ntezurubanza, L. and Svendsen, A. B. (1989). Antimicrobial activities of some *Ocimum species* grown in Rwanda. *Journal of Ethnopharmacology*, 26, 57-63.
- Madeira, S. V. F., Matos, F. J. A., Leal-Cardoso, J. H. and Criddle, D. N. (2002). Relaxant effects of the essential oil of *Ocimum gratissimum* on isolated ileum of the guinea pig. *Journal of Ethnopharmacology*; 81, 1-4.
- Nakamura, C. V., Ueda-Nakamura, T., Bando, E., Melo, A. F. N., Cortez, D. A. G. and Dias, B.P. (1999). Antibacterial activity of *Ocimum gratissimum* L. essential oil. *Mem. Inst. Oswald Cruz*, 94, 675-678.
- Ngassoum, M. B.; Essia-Ngang, J. J.; Tatsadjieu, L. N.; Jirovetz, L.; Buchbauer, G. and Adjoudji, O. (2003). Antimicrobial study of essential Oils of *Ocimum gratissimum* leaves and *Zanthoxylum xanthoxyloides* fruits from Cameroon. *Fitoterapia*, 74, 284-287.
- Offiah, V. N. and Chikwendu, U. A. (1999). Anti-diarrhoea effects of *Ocimum gratissimum* leaf extract in experimental animals. *Journal of Ethnopharmacology*, 68, 327-330.
- Okoli, R., Turay, A.A., Mensah J.K and Aigbe, A.O. (2009). Phytochemical and antimicrobial properties of four Herbs from Edo State Nigeria. Report and opinion 1(5), 1-7.

- Okwu, D.E. and Josiah, C. (2006). Evaluation of chemical composition of two Nigerian medicinal plants. *Afr. J. Biotech.*, 5(4):357-361.
- Orwa, C., Mutua, A.; Kindt, R. ; Jamnadass, R. ; Anthony, S., (2009). *Agro forestry database: a tree reference and selection guide version 4.0*. World Agro-forestry Centre, Kenya; 5 pp.
- Pessoa, L. M.; Morais, S. M.; Bevilaqua, C. M. L. and Luciano, J. H. S. (2002). Anthelmintic Activity Of Essential Oil of *Ocimum Gratissimum* Linn. An Eugenol against *Haemonchus contortus*. *Veterinary Parasitology*, 109, 59-63.
- Pravia, L. D.; Lampman, G.M, and Kriz, G.S., (2001). *Introduction to Spectroscopy, A Guide For Students of Organic Chemistry*. Thompson Learning, Inc. 3<sup>rd</sup> edition, pp 579.
- Winter, E. A. Risley, E.A., and Nuss, G. V. (1963). Anti-Inflammatory and anti-pyretic activities of indomethacin. *Journal of Pharmacology and Experimental Therapeutics*. 141, 369 – 376.
- Wong, S.L., Chang, H.S., Wang, G.J., Chiang, M.Y., Huang, H.Y., Chen, C.H., Tsai, S.C., Lin, C.H., and Chen, I.S. (2011). Secondary metabolites from the roots of *Neolitsea daibuensis* and their anti-inflammatory activity. *Journal of Natural Products* 74: 2489-2496.