# Fatty hydroxamic acid mixture from underutilized *Adansonia digitata* seed oil: a potential means for scavenging free radicals and combating drug resistant microorganisms

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Fatty hydroxamic acids mixture (ADX) was synthesized from underutilized *Adansonia digitata* seed oil (AD). The synthesis was monitored using Fourier Transform Infrared spectrometer (FTIR) and Nuclear Magnetic Resonance ($^1$HNMR). ADX was characterized using X-ray Diffraction analysis (XRD), Scanning Electron Microscopy (SEM), particle size distribution (PSD), zeta potential and thermogravimetric analysis (TGA). ADX was further screened for its antimicrobial activity against certain gram-positive and gram-negative pathogenic organisms as well as for its potential as antioxidant agent. Gas Chromatography (GC) results revealed the most abundant fatty acid in AD to be C18:1 (36.55%). $^1$HNMR confirmed the production of ADX with corresponding peaks. The PSD of ADX was monomodal with a mean size of 0.0541 µm while the zeta potential increased as pH increased. Quantum chemical computational analysis was used to describe the activity of ADX in molecular terms. The lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO) are distributed over the molecules of ADX suggesting its antimicrobial and antioxidant activities may have occurred via donor-acceptor interactions. ADX exhibited antimicrobial activity against all the test pathogenic organisms with the highest activity against *Salmonella enteritidis* at a minimum inhibitory concentration (MIC) of 2.00 µg/mL, which compared better than some previously reported antimicrobial agents in literature. ADX also had an appreciable *in-vitro* antioxidant activity due to its capacity to scavenge DPPH (1,1-Diphenyl-2-picryl-hydroxyl) radical ($IC_{50}$ of 389.12 µg/mL). The present study suggests ADX as an antimicrobial and therapeutic agent for scavenging free radicals and treating drug-resistant pathogens.

**Keywords:** *Adansonia digitata*; Antimicrobial activity; Antioxidant; Fatty acid; DPPH.



## 1. INTRODUCTION

Use of antimicrobial agents has been of great benefit to humans, it has served as means to fight diseases in the past decades. However, pathogenic organisms are developing resistance against the most known antimicrobial agents. Aside from this, some of the conventional synthetic antimicrobial agents produce undesirable side effects [1] and, in some cases, their side products are environmentally non-friendly because they are sometimes non-biodegradable when they get into the environment. The emergence of drug-resistant pathogenic organisms has been of concern, as there is need to develop new antimicrobial agents with higher potency, environmentally friendly and obtained from a renewable source [2].

Traditional medicine has played a key role in primary healthcare in many developing countries especially in the area of infectious diseases. There have been several reports on the use of plant extracts against disease causing organisms with most of these extracts being in the crude forms [3, 4]. It has been shown that natural products from plants may serve as sources of antimicrobial

agents with possible mechanisms to fight drug-resistant pathogens [5]. Some of these plant extracts may require purification, modification or specific co-synthesis in order to improve on their ability to serve this purpose. Plant seed oil belongs to this group of plant extracts that may be chemically modified and screened for the purpose of being antimicrobial [6]. Such modified naturally sourced product may be proposed as a replacement or accompany antibiotics due to the known broad-spectrum antimicrobial activity of potent natural products.

Seed oils have found several applications in the past, some of these applications include the use as medicine [7] but, presently, there is need to develop cheap naturally sourced antimicrobial alternatives that can combat drug-resistant pathogenic organisms. Seed oil is also known to have played important roles in pharmacological research and can be used for the development of novel drugs where they can be used directly as therapeutic agents or as a starting material for the synthesis of drugs and model of pharmacologically active compounds [8]. One of the advantages of using lipid-based drug is their self-emulsifying properties that improves the rate and extent of their absorption into the body when orally administered to sick patients [9]. Previous studies have shown that apart from being antimicrobial, seed oils also have the tendency of being antioxidant [10]. This antioxidant property makes them important in human health such as in the control and management of acute and chronic diseases. The possibility of being antimicrobial and at the same time antioxidant makes seed oil unique in this regard. This capacity of being antimicrobial and antioxidant may be improved by modifying seed oil via a simple reaction route to produce more efficient products. Use of underutilised seed oils is a viable approach to achieving this goal.

*Adansonia digitata* seed oil is an example of underutilised seed oil that may meet this need. The plant is a tree that can grow up to about 5-25 m in height and the trunk can reach a diameter of about 10-14 m. It belongs to the Malvaceae plant family. The seed has been reported to be a good source of oil, phosphorus, calcium and magnesium [11]. Presently, *Adansonia digitata* seed oil is considered to be underutilised in Nigeria since there is no specific use of the oil. The aim of this work is to find application for *Adansonia digitata* seed oil by modifying it into ADX and screening it for its antioxidant and antimicrobial properties against known drug-resistant pathogens.



## MATERIALS AND METHODS

### MATERIALS

The seeds of *Adansonia digitata* were collected from a garden in Ibadan, Oyo State, Nigeria. They were identified at the Department of Botany and Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria. They were air dried at room temperature, ground into powder in an industrial mill and stored in an airtight container for further use. Formic acid, n-hexane, hydrogen peroxide, hydroxylamine hydrochloride, sodium methoxide, methanol and sulphuric acid were purchased from Sigma-Aldrich (Brazil). All other chemicals and reagents used in this study were purchased from Sigma-Aldrich (UK). The powdered seed was later subjected to extraction in a Soxhlet extractor for 10 h using n-hexane in order to obtain the oil as previously described by Adewuyi et al [12].

### FATTY ACID COMPOSITION

Fatty acids composition of AD was determined as fatty methyl esters as previously described by Adewuyi et al [12] with little modifications. Fatty methyl esters were prepared by refluxing the oil at 70°C for 3 h in 2 % sulphuric acid in methanol. Estimation and identification of the respective fatty acids was carried out on a HP7820A gas chromatograph (Agilent) equipped with flame ionization detector on a split injector with a split ratio 50:1. A SP2560 column (30 m × 0.25 mm × 0.20 µm) was used with the injector and detector temperature maintained at 250°C and 260°C, respectively. The oven temperature was programmed at 120°C with a steady increase at 7°C /min until this was finally increased to 240°C. The carrier gas was hydrogen at a flow rate of 1.5 mL/min and the volume of injection was 1 µL. Data acquisition program EZChrom Elite Compact (Agilent) was used while the identification of peaks was made by comparison with standards of fatty acid methyl esters (Supelco cat no 18917).

### CONVERSION OF AD TO ADE (METHYL ESTERS)

This was achieved stepwise. A two-step acid–base catalysed transesterification was conducted in order to assure the conversion of total fatty acids to methyl esters (including free fatty acids). Firstly, AD was treated with 2% sulphuric acid in methanol at 70°C for 2 h under reflux and continuous stirring in order to convert any available free fatty acid in AD to methyl esters. After this, the treated AD was further subjected to transesterification using 1% KOH in methanol at 70°C for 4 h. The final mixture obtained was extracted with ethyl acetate, washed severally with distilled water until free of KOH and passed over sodium sulphate. Ethyl acetate was removed from the ethyl acetate extract using a rotary evaporator in order to obtain the ADE.

### HYDROXYLATION OF ADE

ADE was hydroxylated as previously described [13]. Briefly, ADE (0.0482 mol) and formic acid (0.106 mol)

were poured into a three-necked round-bottom flask and cooled down to a temperature of 15°C while continuously stirred under reflux. Hydrogen peroxide (0.407 mol) was further added to the flask drop wise for about 20 min. The reaction temperature was later raised to 70°C and maintained at this temperature for 18 h. The mixture was cooled to room temperature and the ADH (hydroxylated ADE) produced was extracted with ethyl acetate, washed severally with distilled water until it was acid free and passed over sodium sulphate. Ethyl acetate was later removed from the final product using a rotary evaporator in order to obtain the ADH.

## SYNTHESIS OF ADX

To achieve this, ADH (2.5 g), methanol (50 mL) and hydroxylamine hydrochloride (3.75 g) were poured into a 250 mL Erlenmeyer round bottom flask. The mixture was stirred for 5 min, sodium methoxide (0.85 g) was added and the pH was maintained above pH 10 using few drops of 0.5 M NaOH. The temperature was gradually increased to 70°C while being stirred continuously for 45 min to give the resulting solid that was filtered and dried in an oven at 60°C. The yield of ADX was found to be 96% (wt/wt). The reaction scheme is as shown in Scheme 1.

## FTIR SPECTROSCOPY

The functional groups in AD, ADE, ADH and ADX were determined using FTIR (Perkin Elmer, spectrum RXI 83303). The samples were blended with KBr, pressed into pellets and analysed in the range of 400 - 4500 $cm^{-1}$.

## $^1$HNMR

$^1$HNMR spectra of AD, ADE, ADH and ADX were recorded on a 200 MHz/52 MM Bruker B-ACS 120 NMR spectrophotometer in $CDCl_3$ containing some amount of TMS as internal standard.

## X-RAY DIFFRACTION ANALYSIS

The X-ray diffraction pattern of ADX was obtained using X-ray diffractometer (XRD-7000X-Ray diffractometer, Shimadzu) with filtered Cu Kα radiation operated at 40 kV and 40 mA. The XRD pattern was recorded from 10 to 80° (2θ), with a scanning speed of 2.00° per minute.

## THERMOGRAVIMETRIC ANALYSIS (TGA)

Thermal stability and fraction of volatile components of ADX were monitored by DTA-TG apparatus (SHIMADZU, C30574600245). This was carried out under nitrogen atmosphere.

## PARTICLE SIZE DISTRIBUTION (PSD) AND ZETA POTENTIAL

ADX was analysed for PSD and zeta potentials using a zeta potential analyser (DT1200, Dispersion technology) at 25°C while observing general calculation model for irregular particles on AcoustoPhor Zetasize 1201 software (version 5.6.16).

## SCANNING ELECTRON MICROSCOPY (SEM)

The surface morphology of ADX was studied using FEI quanta 200 (model EDAX EDS) operated on Genesis software, version 5.21. ADX was prepared for SEM analysis by coating its surface with gold in order to increase electrical conductivity and to improve the quality of the micrographs.

## ANTIOXIDANT ACTIVITY ASSAY

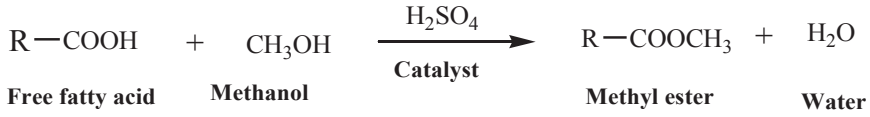
The antioxidant activity of ADX was determined by measuring its DPPH free radical scavenging capacity, which was achieved spectrophotometrically according to Tahir et al [14] but with slight modification. Stock solution of DPPH (12.5 mg in 50 mL of methanol) was prepared and kept away from light. The prepared DPPH (1 mL) was added to various concentrations of ADX (500, 250, 200, 100 and 50 µg/mL) in methanol. This was incubated at 37°C in the dark for 30 min. Thereafter, the absorbance of the assay mixture was determined at 517 nm. Ascorbic acid (500, 250, 200, 100 and 50 µg/mL) was used as standard. A blank experiment (without ADX) as control was carried out. This was also incubated at 37°C in the dark for 30 min and the absorbance read at 517 nm. The reaction was recorded in triplicate. Scavenging activity was calculated as follows

$$Q\ (\%) = = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

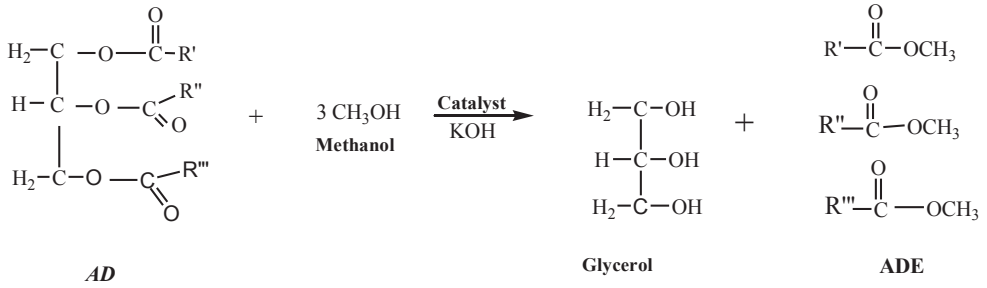
## ANTIMICROBIAL SCREENING

ADX was screened against the following organisms: *Salmonella typhi* (Clinical sample), *E-coli* (ATCC 29522), *Staphylococcus aureus* (ATCC 700699), *Klebsiella pneumoniae* (ATCC 8309), *Bacillus subtilis* (ATCC 6633), *Shigella flexneri* (ATCC 120222) and *Salmonella enteritidis* (ATCC 1307). The organisms were obtained from Microbiology Laboratory of the Department of Biological Sciences, Redeemer's University, Ede, Nigeria where they are kept for experimental purposes. The screening was achieved using the agar well diffusion method on Mueller-Hinton agar. Briefly, Mueller-Hinton agar plates were inoculated with bacterial strain under aseptic conditions. Wells were bored using a cock borer (diameter of 6

***1 - Esterification of free fatty acid in AD with 2% $H_2SO_4$ / MeOH***



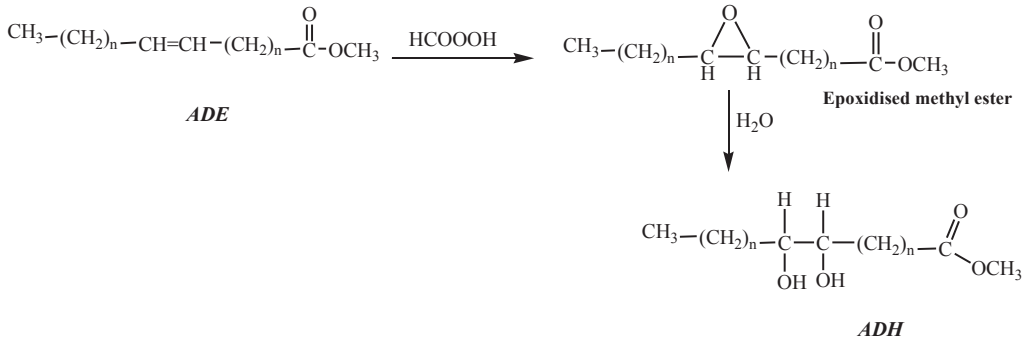

***2 - Transesterification of AD to ADE using 1% KOH / MeOH***



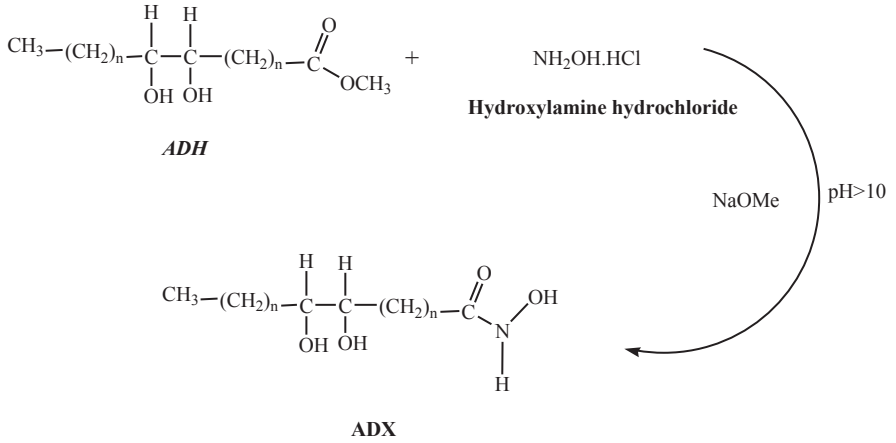

**3 - *Hydroxylation***

*First step: Formation of Performic acid*


*Second step: Hydroxylation reaction*




***4 - Synthesis of ADX***




**Scheme 1 -** Synthesis of ADX

mm); these were filled with 50 µL of ADX and incubated at 37°C for 24 h. The diameter of the growth inhibition zones was measured after the incubation. The controls were the oil (AD) which was the starting material used in the synthesis of ADX and distilled water, which was used to dissolve ADX (300 µg/mL). Ciprofloxacin was used as the positive control at a concentration of 300 µg/mL.

### DETERMINATION OF MIC

The MIC value of ADX was determined as the lowest concentration that completely inhibited bacterial growth after 48 h of incubation at 37°C. Using wire loop, the cultures were aseptically cultured in nutrient agar for 24 h at 37°C, transferred into sterile nutrient broth while standardisation of cells was adjusted to $1\times10^{-6}$ cell/mL with the aid of haemocytometer. The standardised culture was pipetted (1 mL) and poured unto readily prepared plate of Mueller Hinton Agar where they were spread on the plate. Holes of 6 mm at about 8 mm depth were bored with the aid of cock borer and filled with 50 µL of aqueous concentrations (ranging from $2 \times 10^{3} – 2 \times 10^{-7}$ µg/mL), of ADX. The MIC value of ADX was determined after 48 h.

### QUANTUM CHEMICAL PARAMETERS

All theoretical calculations were performed using the DFT electronic structure programs at B3LYP/6-31G level theory using Spartan 14.1 software. The molecular electronic structure of three most abundant constituents (C16:0 fatty hydroxamic acid, hydroxylated C18:1 fatty hydroxamic acid and hydroxylated C18:2 fatty hydroxamic acid) of ADX were modelled. This also included the distribution of frontier molecular orbitals in order to establish the reactivity of ADX with free radicals and pathogenic microorganisms as well as its active sites for the antioxidant and antimicrobial processes.

## RESULTS AND DISCUSSION

### CHARACTERISATION

The oil yield of *Adansonia digitata* seed was 19.64%. The fatty acid composition of the oil is presented in Table I. The most abundant fatty acid in the oil is C18:1 (36.55%) followed by C18:2 (28.19%). The abundance of these two unsaturated fatty acids is an indication of high unsaturation in the oil that was the reason for improving on its property via hydroxylation of the double bonds in it. The FTIR reveals the different functional groups in the samples as shown in Figure 1. The peaks at 2962 and 2892 $cm^{-1}$ accounts for the C-H stretching of $–CH_3$ and $–CH_2$, respectively while peaks seen at 3005 $cm^{-1}$ in AD and ADE were attributed to the C-H stretching of -C=C- suggesting the presence of unsaturation in AD and ADE. This peak at 3005 $cm^{-1}$ in AD and ADE was not found in ADH suggesting the fact that hydroxylation reaction took place at the unsaturation region (-C=C-) of ADE. The disappearance of the unsaturation peak at 3005 $cm^{-1}$ gave rise to the peak at 3442 $cm^{-1}$ in ADH, which may be considered as being the vibrational frequency of the OH group. The band at 1748 $cm^{-1}$ in AD, ADE and ADH was attributed to the vibrational frequency of the ester carbonyl group. The peak at 1748 $cm^{-1}$ was not found in ADX suggesting the conversion of the ester group to hydroxamic acid with the appearance of peaks at 1665 and 1564 $cm^{-1}$ that were attributed to carbonyl group of hydroxamic acid and the bending of the CNH group, respectively. The overlap of the NH stretching and the OH group was seen at 3455 $cm^{-1}$ while the peak at 1425 $cm^{-1}$ was assigned to the in plane bending of N-O-H group in ADX [15].

**Table I -** Fatty acid composition of AD

| Fatty acid | Composition (%) |
|---|---|
| C14:0 | 0.31 ± 0.10 |
| C16:0 | 22.20 ± 0.05 |
| C16:1 | 0.25 ± 0.03 |
| C18:0 | 4.41 ± 0.10 |
| C18:1 | 36.55 ± 0.05 |
| C18:2 | 28.19 ± 0.10 |
| C18:3 | 0.33 ± 0.10 |
| C20:0 | 0.99 ± 0.05 |
| Others | 6.77 ± 0.07 |

The $^{1}$HNMR result in Figure 2 reveals the methylene protons of AD, ADE, ADH and ADX within the range 1.1-1.5 ppm while the proton of terminal methyl groups was seen at 0.8 ppm. The signal at 4.0-4.3 ppm in AD was considered as being due to the glyceridic methylene protons, which was not found in other spectra confirming the transesterification of the triglyceride to fatty methyl esters. The peak at 5.3 ppm in AD and ADE confirms the presence of unsaturation that was attributed to the olefinic protons, that appeared at 3005 $cm^{-1}$ in the FTIR spectra. This peak was not found in ADH and ADX indicating that the unsaturated bonds had taken part in hydroxylation reaction. The ester peak was seen at 2.8 ppm in AD but shifted to 3.5 ppm in both ADE and ADH. This observation may be due to the hydrolysis of the triglyceride backbone during transesterification. The ester peak was not found in ADX indicating conversion to hydroxamic acid. The alkyl chain hydroxyl group proton appeared at 3.8 ppm in ADH and at 3.4 ppm in ADX. The formation of ADX was confirmed with the appearance of peaks at 3.6 ppm (N-OH) and at 7.3 ppm (N-H).

The XRD diffraction pattern and the thermal stability of ADX are presented in Figure 3. The diffraction pattern is similar to what was previously reported by Zabar et al [16] for linoleic acid complex. Regard-

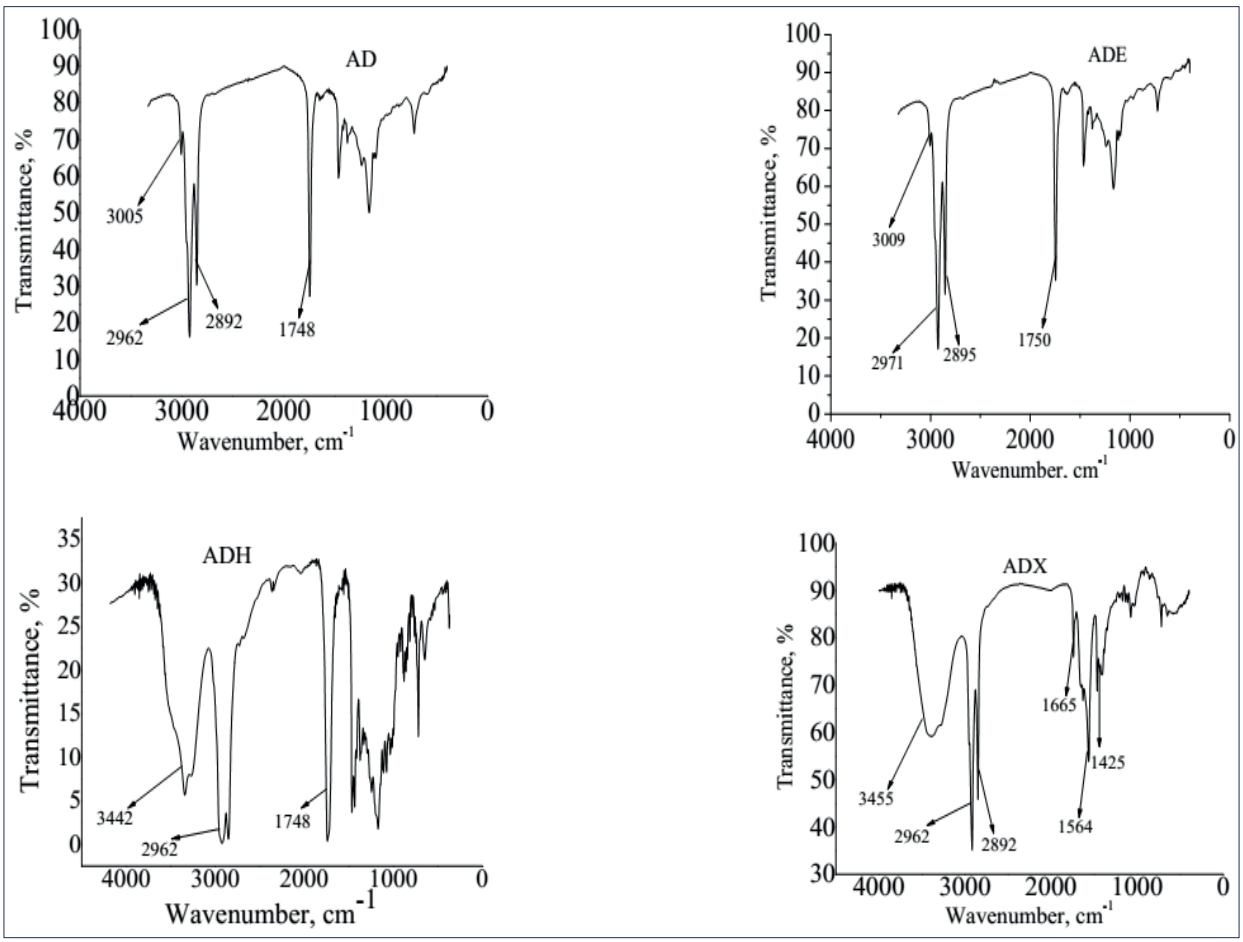



**Figure 1 -** FTIR of AD, ADE, ADH and ADX



ing the TG result, an initial loss in weight was seen at 100°C, which may be attributed to loss of water molecules absorbed by ADX. This was followed by a weight loss at around 230-350°C. Loss in weight at around 360-500°C may be attributed to a loss of the alkyl carbon chain length of the constituent fatty acids while loss in mass above 500°C may be due to loss of char. The PSD and zeta potential of ADX is presented in Figure 4. The PSD is monomodal with a mean size of 0.0541 µm. The zeta potential increased as pH increased. The average zeta potential presents ADX to be electrically stabilised with a minimal tendency of coagulating or flocculating in solution [17]. As shown in Figure 5, the SEM of ADX revealed a homogeneous, jelly and amorphous surface.

## ANTIOXIDANT ACTIVITY ASSAY

Antioxidants are molecules that inhibit the oxidation of other molecules. Most times, they prevent or terminate oxidation reactions in biological systems preventing the formation of free radicals that may damage the cells. Different mechanisms of action have been reported for free radical scavenging antioxidants, these include direct neutralization throughout oxidation-reduction reactions, induction of the expression of antioxidant enzymes or inhibition of pro-oxidant enzymes [18]. Presently, ADX was evaluated for its capacity to play the role of antioxidants. It was observed that ADX exhibited an antioxidant capacity. This was determined as its ability to scavenge free radicals. The capacity of ADX to scavenge free radicals was found to increase as its concentration reduces, as shown in Table II. The highest scavenging activity was 93.09% at a concentration of 50 µg/mL while the least activity was 26.24% at a concentration of 500 µg/mL with no activity observed at 1000 µg/mL. This is an indication that ADX may exert a pro-oxidant activity at high concentration. Similar observation had been reported by Adewuyi et al [19] using *Cyperus esculentus* seed oil as source of hydroxamic acid. This activity may be due to the ability of ADX to have neutralised the free radical character of DPPH by transferring either electron or hydrogen to DPPH since ADX has heteroatoms (nitrogen and oxygen) in its molecule [20, 21]. The scavenging capacity of ADX was lower than that of vitamin C. The concentration of ADX, required to achieve a 50% reduction in DPPH radicals ($IC_{50}$), was calculated using the concentration vs activity curve. The $IC_{50}$ for ADX was found to be 389.12 µg/mL. This value was compared with that of

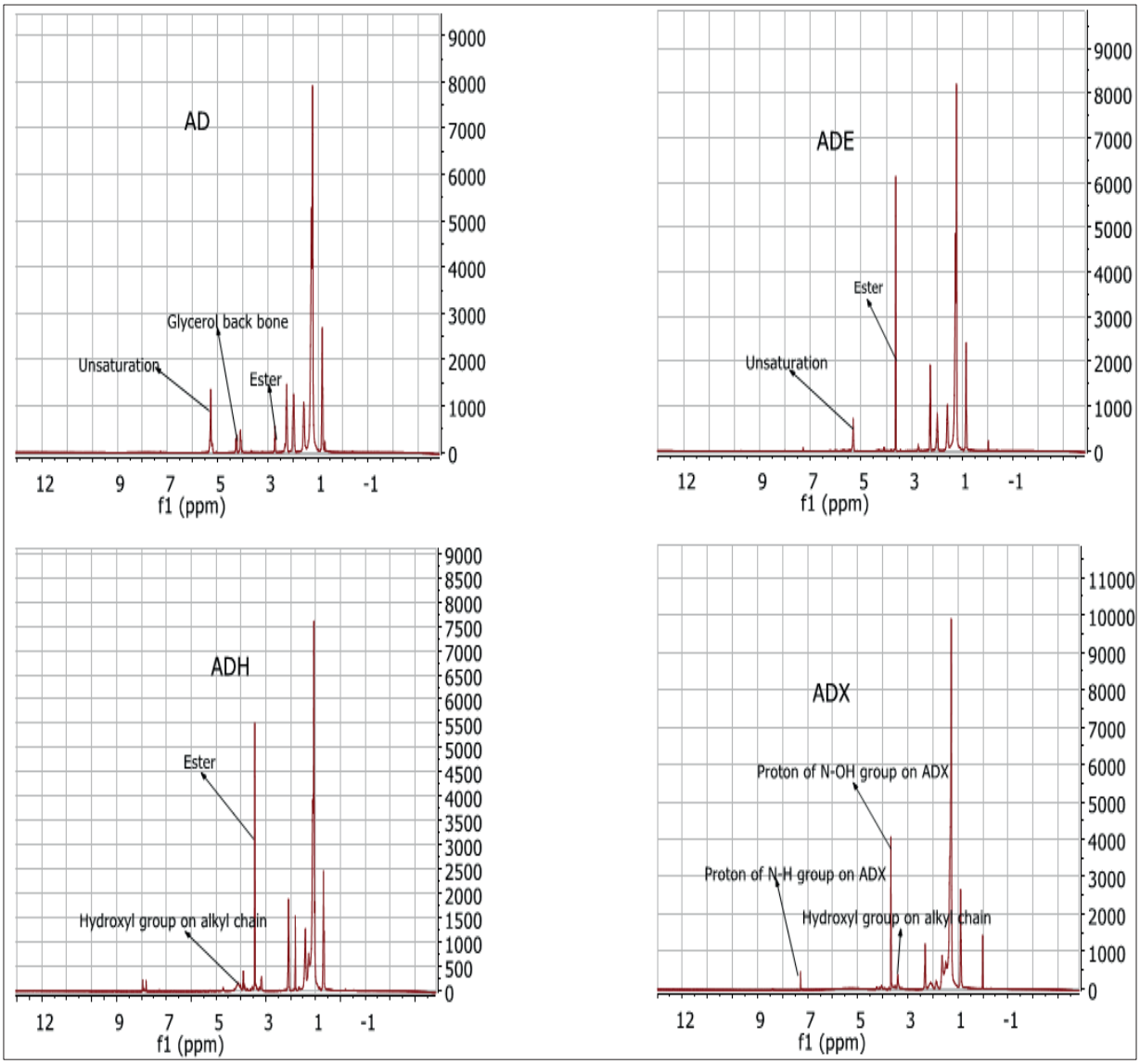



**Figure 2 -** $^{1}$HNMR of AD, ADE, ADH and ADX

a known antioxidant; in this case, vitamin C was used as a known antioxidant. The $IC_{50}$ of vitamin C was calculated to be 151.98 µg/mL. Although vitamin C has high $IC_{50}$ value, but it has been known to exhibit drug-drug interaction when used along with some drugs during treatment of certain ailment [22]. When

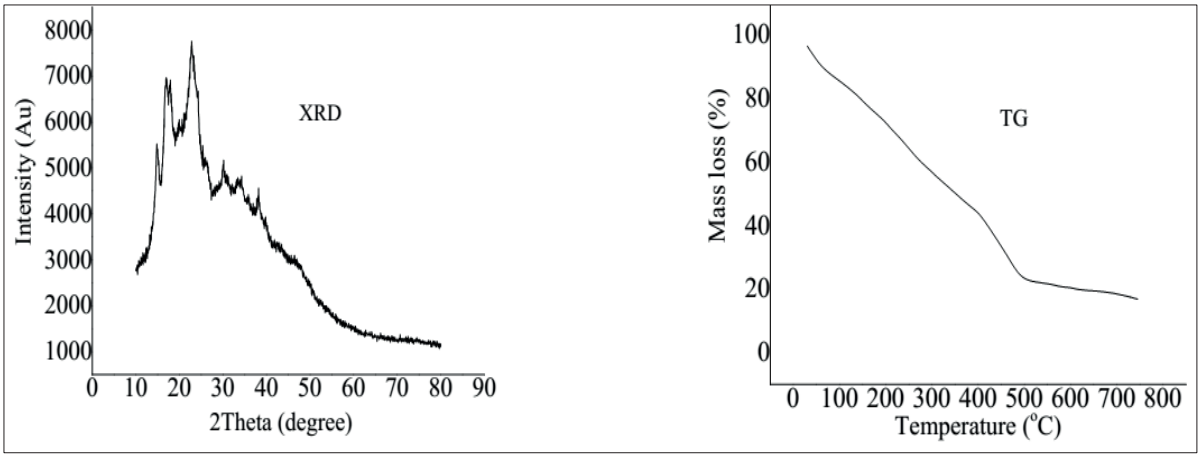



**Figure 3 -** The XRD and TG results of ADX

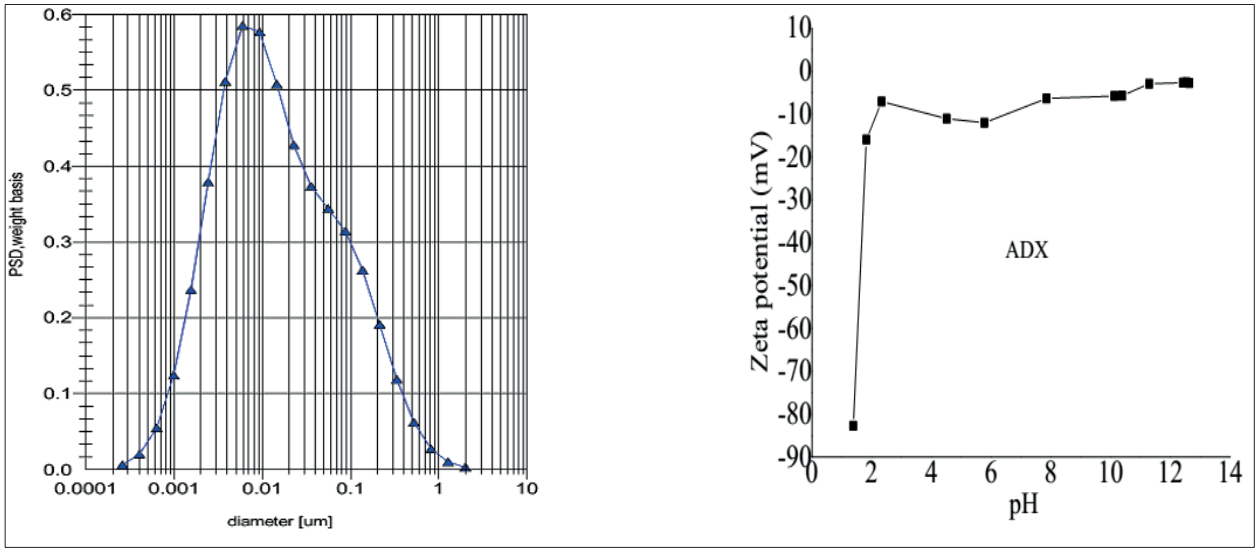



**Figure 4 -** PSD and zeta potential of ADX

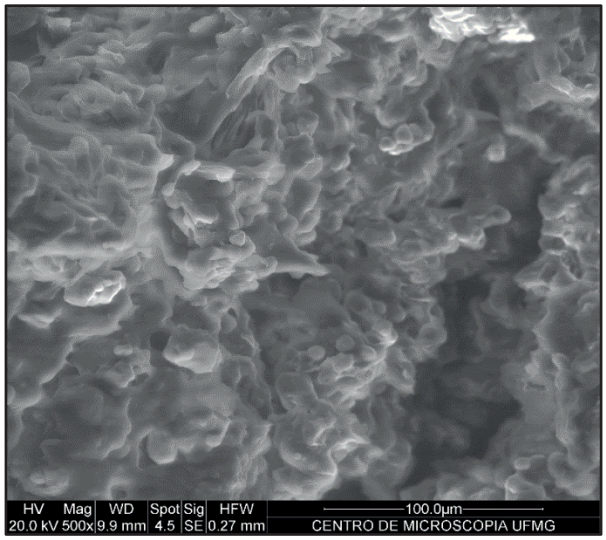



**Figure 5 -** Micrograph of ADX

**Table II -** DPPH assay for the scavenging activity of ADX and vitamin C

| ADX (µg/mL) | Q (%) | Vitamin C |
|---|---|---|
| 1000 | - | - |
| 500 | 26.24 ± 0.00 | 97.79 ± 0.02 |
| 250 | 87.02 ± 0.02 | 91.71 ± 0.01 |
| 100 | 92.54 ± 0.01 | 97.79 ± 0.02 |
| 50 | 93.09 ± 0.04 | 98.07 ± 0.05 |

- = No scavenging activity
Q = Scavenging activity

treating certain ailments like a viral infection, vitamin C is included in medication to boost immunity; however, some drugs used in a combined therapy have the capacity of reacting with vitamin C (drug-drug interaction) in the body system. Such drug-drug interaction might be due to reactions between the function groups of these drugs when ionised in the body system. This interaction may sometimes hamper the efficacy of such drugs; that calls for replacement or alternative to vitamin C under such peculiarities. It is hoped that ADX may be able to meet this need, but further detailed research work will be required in this regard. Although previous work on toxicity studies revealed that hydroxamic acid had a promising safety profile when fed to Wistar rats [19] but there is need for more evaluation to ascertain this.

## ANTIMICROBIAL SCREENING AND MIC

ADX was screened against seven organisms as shown in Table III. The antimicrobial activity was estimated by means of zone of inhibition. The controls (AD and water) did not exhibit any antimicrobial activity. At a concentration of 200 µg/mL, ADX exhibited inhibition against all the test organisms. ADX had the highest inhibition against *Salmonella enteritidis* (15 mm), a gram-negative pathogenic bacterium mostly known for food poisoning. The lowest inhibition was found against *Salmonella typhi* (6 mm), *Staphylococcus aureus* (6 mm) and *Klebsiella pneumonia* (6 mm), that have been reported as a multidrug resistant pathogen [23, 24]. Although several drugs or antimicrobial agents have been developed in the past but overtime, these organisms have been developing resistance against the efficacy of some of these drugs or antimicrobial agents. *Salmonella typhi* is known to be responsible for typhoid fever, that has caused serious public health problems in most African countries especially in Nigeria. The major concern is its changing mode of presentation and multidrug resistance towards established drugs like trimethoprim-sulfamethoxazole, chloramphenicol and ampicillin [25, 26],

**Table III -** Antimicrobial screening of ADX

| Organism | Inhibition (mm) | Ciprofloxacin (mm) |
|---|---|---|
| *Salmonela typhi* | 6.00 ± 0.05 | 35.00 ± 0.00 |
| *E.coli* | 12.00 ± 0.01 | 29.00 ± 0.00 |
| *Staphylococcus aureus* | 6.00 ± 0.03 | - |
| *Klebsiella pneumoniae* | 6.00 ± 0.10 | 36.00 ± 0.00 |
| *Bacillus subtillis* | 11.00 ± 0.01 | 26.00 ± 0.00 |
| *Shigella flexneri* | 12.00 ± 0.10 | 30.00 ± 0.00 |
| *Salmonella enteritidis* | 15.00 ± 0.05 | 9.00 ± 0.01 |

- = No inhibition

that makes it difficult to diagnose and treat. In this study, ADX exhibited ability to inhibit the growth of this dangerous pathogen. The MIC of ADX was compared with other antimicrobial agents from plant extracts reported in literature as presented in Table IV. The MIC value obtained for *Salmonela typhi* (20.00 µg/mL) compared favourably with values reported for known drugs such as trimethoprim-sulfamethoxazole [26]. The value also compared better than values reported for some plant extracts [27]. *E. coli* is one of the most common gram-negative pathogens in humans and has been reported with multidrug resistant strains transferable to other strains with high potential of posing a serious health risk to humans [28]. A study conducted in Nigeria has shown resistance of *E. coli* to some antibiotics, which included ciprofloxacin [29]. The MIC obtained for ADX (200.00 µg/mL) against *E. coli* in this study is higher than the value reported for cefoxitin (16 µg/mL) in the previous study [30]. Other reports such as compounds isolated from *Nauclea pobeguinii* [31], some medicinal plant extract [32] and *Piper betle* [33] showed MIC values higher than that of ADX toward *E. coli*. *Staphylococcus aureus* is a gram-positive bacterium that causes diseases in human with leading cause of skin and soft tissue infections. The MIC result of ADX against *Staphylococcus aureus* was found as better than that reported for green tea extract [34]. The value also compared better than those reported for *Saururus chinensis and Geum japonicum* [35] in Table IV. *Klebsiella pneumoniae* are gram-negative bacteria capable of causing a respiratory infection. ADX exhibited a MIC of 200.00 µg/mL, against *Klebsiella pneumoniae*. The value was found as lower than the value reported for *Psidium guajava* [33], *Azadirachta indica* [36] and *Houttuynia cordata* [35]. ADX has a MIC of 20.00 µg/mL against the growth of *Bacillus subtillis*. This value is lower and compared better than values previously reported by Garmana et al [37] for *T. tuberculata*, *A. cordifolia*, *K. galanga*, *C. zedoaria*, *Z. officinale* and *M. citrifolia*. Gastrointestinal and enteric diseases continue to be a major problem in the world with the greatest disease burden being in the sub-Saharan Africa and Asia [38, 39]. This problem is caused by certain gastroenteritis bacterial with Shigella playing a big role in epidemiology of diarrhoea [39, 40]. The MIC value of ADX against *Shigella flexneri* was found to be 200.00 µg/mL, which is better than the value reported by Farooqui et al [41] for *Camellia sinensis*. This value of 200.00 µg/mL, is lower than values reported by Bisi-Johnson et al [39] for *Acacia mearnsii*, *Eucomis comosa* and *Pelargonium*



**Table IV -** Comparison of the minimum inhibitory concentration of ADX with other antimicrobial agents in literature

| Organism | SC | GJ | AP | HC | PG | SA | AA | AI | BV | ADX |
|---|---|---|---|---|---|---|---|---|---|---|
| *Salmonella typhi* | - | - | - | - | - | - | - | - | - | 20.00 |
| *E.coli* | 312.50 | 78.12 | 1250.00 | 1250.00 | | | 3410.00 | 4270.00 | 4270.00 | 200.00 |
| *Staphylococcus aureus* | 2500.00 | 2500.00 | - | - | 1250.00 | 1250.00 | 290.00 | 3410.00 | 3410.00 | 200.00 |
| *Klebsiella pneumoniae* | 39.00 | 78.12 | 1250.00 | 1250.00 | - | - | 670.00 | 4270.00 | 4270.00 | 200.00 |
| *Bacillus subtillis* | 1250.00 | 625.00 | 2500.00 | 1250.00 | - | - | - | - | - | 20.00 |
| *Shigella flexneri* | - | - | - | - | - | - | - | - | - | 200.00 |
| *Salmonella enteritidis* | 1250.00 | 78.12 | 1250.00 | - | | | | | | 2.00 |

- = Not determined

Values are in µg/mL

SC = *Saururus chinensis [35], GJ = Geum japonicum [35], AP = Agrimonia pilosa [35], HC = Houttuynia cordata [35], PG = Punica granatum* [37], SA = *Syzygium aromaticum* [37], AA = *Anogeissus acuminate* [36], AI = *Azadirachta indica* [36], BV = *Bauhinia variegate* [36]

*sidoides* but higher than values for *Aloe arborescence* and *Aloe striata*. This suggests that ADX may be used as alternative remedy for stomach related ailments. *Salmonella enteritidis* has been closely associated with human and other lower animals. It is separated from other salmonella species because it is specifically cited in zoonosis and moreover, it's epidemiological different when compared to other salmonellae. The MIC for *Salmonella enteritidis* was found to be 2.00 µg/mL, which compares favourably with values obtained for *Agrimonia pilosa* and *Geum japonicum* [35]. The value is also higher than values reported by Voss-Rech et al [42] for *Achyrocline satureioides*, *Eugenia jambolan* and *Eugenia uniflora*.

Ciprofloxacin that was used as a positive control showed higher zones of inhibition than ADX for the test organisms except for *Salmonella enteritidis* and *Staphylococcus aureus*. Studies have revealed that these organisms are now developing resistance against ciprofloxacin and other known conventional drugs. Report from Nepal has shown resistance of *Salmonela typhi* against ciprofloxacin [43]. Similar observation but of higher *Salmonella typhi* resistance was also reported from Bangladesh against ciprofloxacin [44]. The ability of these organisms to multi-resist drugs has been reported by Dever and Dermody [45] to be through three major mechanisms, which may be via: changes in membrane permeability to antibiotics, enzymatic degradation of antibacterial drugs and alteration of bacterial proteins that are antimicrobial targets. There is the possibility of encountering untreatable diseases in the near future due to a multidrug resistance pattern of some of these pathogens that suggests the need for the synthesis of alternative drugs to replace conventional drugs that these organisms have developed resistance against. This study has revealed ADX as a potential antimicrobial agent that might be useful in circumventing this problem as a direct agent or co-agent against these organisms. There may be a need for further study on the toxicity and safety profile of ADX.

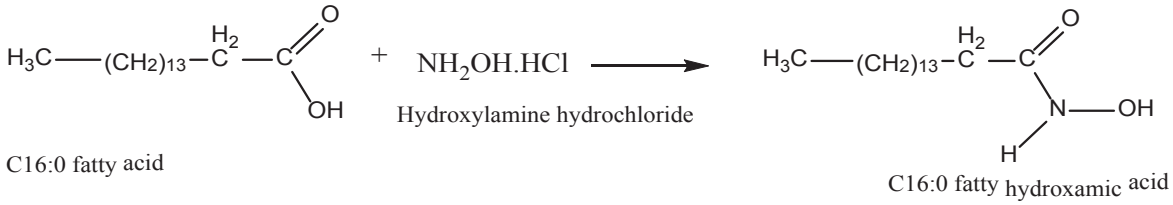


## QUANTUM CHEMICAL PARAMETERS

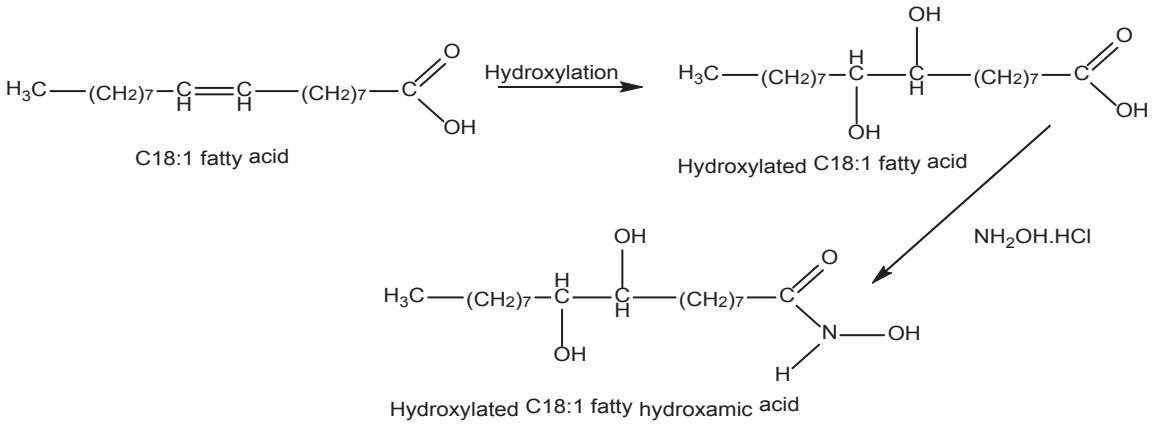
The quantum chemical computational analysis helps in describing and understanding the interaction between ADX, free radicals and pathogenic microorganism in molecular terms. The GC results revealed C16:0, C18:1 and C18:2 as the major constituents of the starting material (AD) that suggested that the major constituents of ADX are C16:0 fatty hydroxamic acid, hydroxylated C18:1 fatty hydroxamic acid and hydroxylated C18:2 fatty hydroxamic acid. The scheme for their synthesis is described in Scheme 2. To this effect, DFT was applied in describing the electronic structural changes that occurred during the antioxidant and antimicrobial processes. This study used DFT to describe the theoretical framework in identifying the contributions of ADX antioxidant and antimicrobial mechanisms and strength. DFT was performed at B3LYP/6-31G level theory using Spartan 14.1 software. The geometry optimisation was followed by modelling the molecular electronic structures and the distribution of frontier molecular orbitals in order to establish the reactivity of ADX as well as its active sites. Figures 6-8 reveals the optimised geometry, HOMO density distribution, LUMO density distribution, total electron distribution, electrostatic potential map and local ionisation potential map of the three major constituents of ADX that are C16:0 fatty hydroxamic acid, hydroxylated C18:1 fatty hydroxamic acid and hydroxylated C18:2 fatty hydroxamic acid.

As shown in the electronic structural changes, the HOMO and LUMO are distributed over the molecule of C16:0 fatty hydroxamic acid, hydroxylated C18:1 fatty hydroxamic acid and hydroxylated C18:2 fatty hydroxamic acid. This may be due to the presence of lone pairs of electrons from oxygen and nitrogen atoms in their molecules. HOMO, which is the region of highest electron density, are possible sites where an electrophilic attack occurs. Such sites are the active centres with the utmost energy to bond with free radicals and the cells of the studied pathogenic microorganisms. In this case, the LUMO orbital of C16:0 fatty hydroxamic acid, hydroxylated C18:1 fatty hydroxamic acid and hydroxylated C18:2 fatty hydroxamic acid accepts electrons from the molecular orbital of the free radicals as DPPH, as well as from the constituent of the cells (enzymes, cellular membrane, DNA, etc) of the pathogenic microorganisms using antibonding orbitals to form feedback bonds. This interaction with the constituents of the cells of the pathogenic microorganisms may have plasmolysed the cells resulting in the death of these organisms or may have distorted the cell constituents that may affect cellular activities and, as a result, causing the observed growth inhibition. This interaction can also be viewed as a reaction between ADX and the cellular protein or enzymes of the microorganisms as previously described by Costa et al [46].

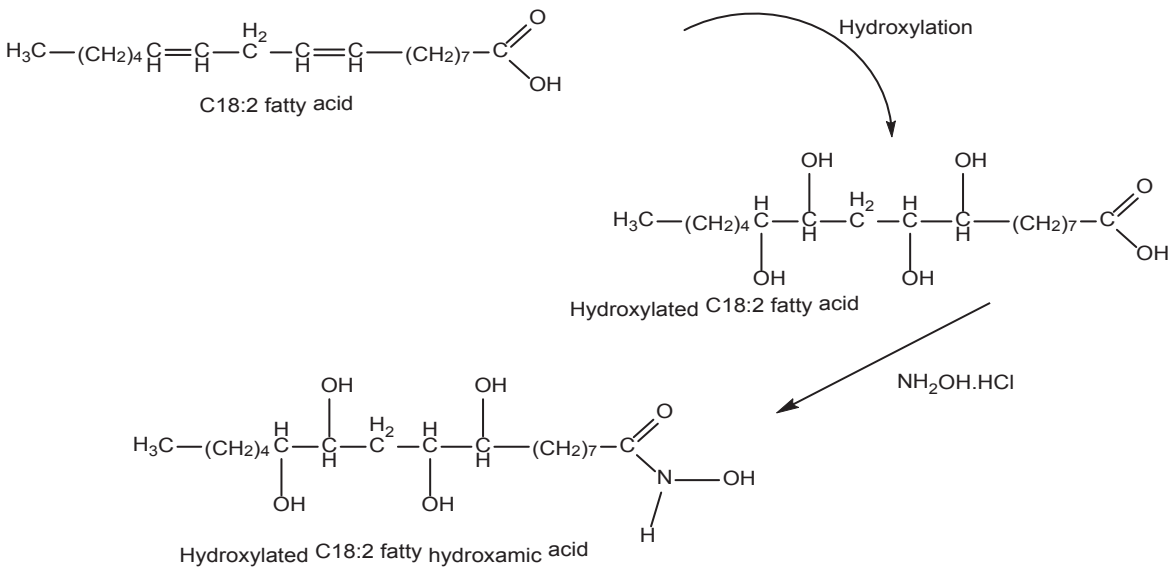
The heteroatoms (nitrogen and oxygen atoms) in ADX are most probably the reactive site for the observed activities that further suggests the reaction between ADX and pathogenic microorganisms/free radicals to have occurred via donor-acceptor bonding among lone pairs of electron and the electronic orbitals of free radicals (DPPH) as well as the cellular constituents of the microorganisms. Mulliken atomic charges revealed the surfaces of the heteroatoms to be negatively charged and that is an indication that these atoms are the active sites for the bonding with free radicals as well as the microorganism cells. The more negatively charged, these atoms (active sites) are the more available for bonding with the free radicals and the cells of the microorganisms. This also suggests how easily the active sites donate their electrons to




**Scheme 2A -** C16:0 fatty hydroxamic acid




**Scheme 2B -** Hydroxylated C18:1 fatty hydroxamic acid




**Scheme 2C -** Hydroxylated C18:2 fatty hydroxamic acid

**Scheme 2 -** Description of the synthesis of the three major constituents of ADX

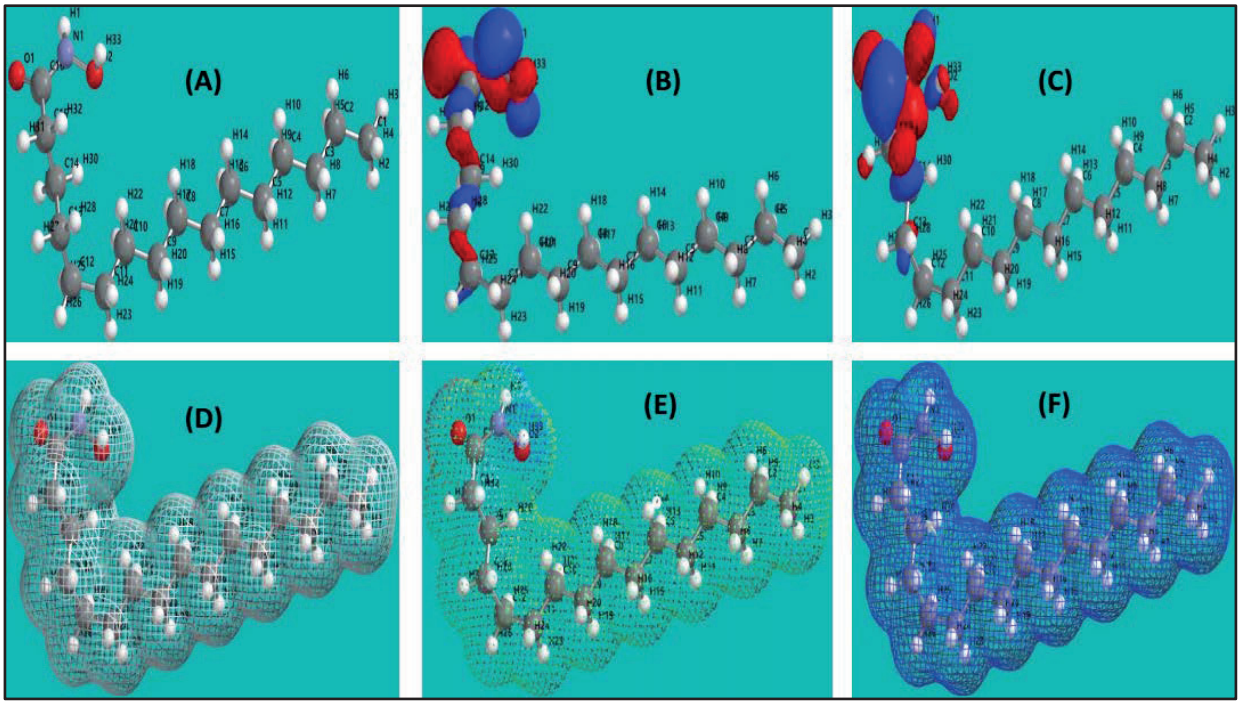



**Figure 6 -** Electronic properties of C16:0 fatty hydroxamic acid. (A): Optimized structure of C16:0 fatty hydroxamic acid, (B): HOMO density distribution of C16:0 fatty hydroxamic acid, (C): LUMO density distribution of C16:0 fatty hydroxamic acid, (D): Total electron density of C16:0 fatty hydroxamic acid, (E): Electrostatic potential map of C16:0 fatty hydroxamic acid, (F): Local ionization potential map of C16:0 fatty hydroxamic acid



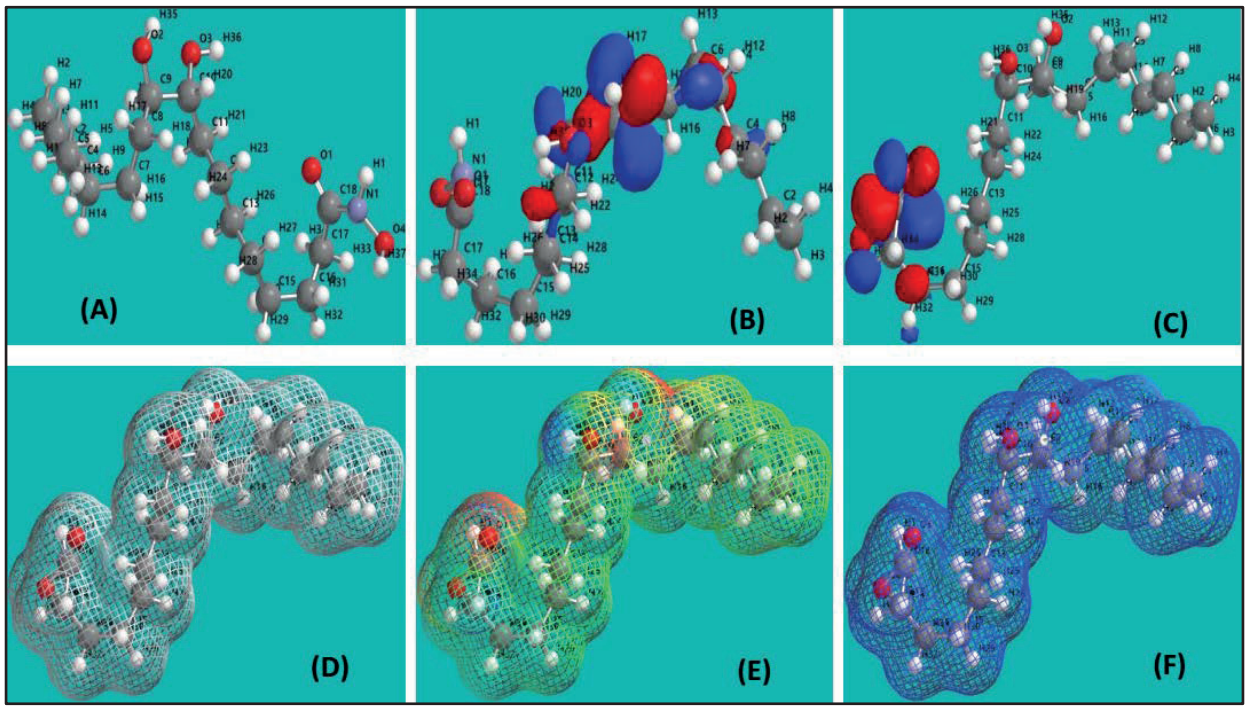



**Figure 7 -** Electronic properties of hydroxylated C18:1 fatty hydroxamic acid. (A): Optimized structure of hydroxylated C18:1 fatty hydroxamic acid, (B): HOMO density distribution of hydroxylated C18:1 fatty hydroxamic acid, (C): LUMO density distribution of hydroxylated C18:1 fatty hydroxamic acid, (D): Total electron density of hydroxylated C18:1 fatty hydroxamic acid, (E): Electrostatic potential map of hydroxylated C18:1 fatty hydroxamic acid, (F): Local ionization potential map of hydroxylated C18:1 fatty hydroxamic acid

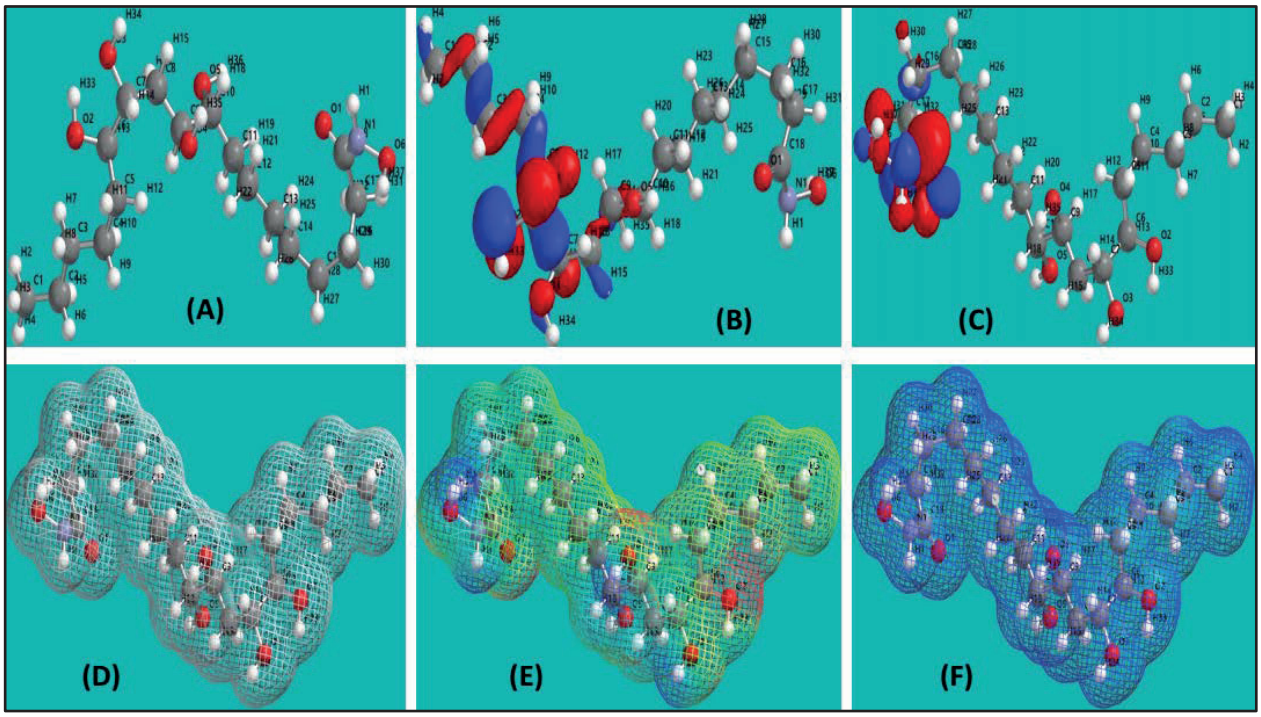



**Figure 8 -** Electronic properties of hydroxylated C18:2 fatty hydroxamic acid. (A): Optimized structure of hydroxylated C18:2 fatty hydroxamic acid, (B): HOMO density distribution of hydroxylated C18:2 fatty hydroxamic acid, (C): LUMO density distribution of hydroxylated C18:2 fatty hydroxamic acid, (D): Total electron density of hydroxylated C18:2 fatty hydroxamic acid, (E): Electrostatic potential map of hydroxylated C18:2 fatty hydroxamic acid, (F): Local ionization potential map of hydroxylated C18:2 fatty hydroxamic acid

orbitals of the free radicals and cells of the microorganisms to exhibit their antioxidant and antimicrobial properties, respectively. The molecular properties for the three main constituents are shown in Table V. The ability of ADX to function as an antioxidant and antimicrobial agent may be related to frontier molecular orbitals. The highest HOMO energy exhibited by hydroxylated C18:1 fatty hydroxamic acid may mean higher electron donating capacity to low energy molecular orbital of the free radicals and the cells of the microorganisms when compared with the other major constituents of ADX. However, the LUMO energy is considered as the susceptibility of ADX molecules to nucleophilic attack and electron affinity. This means that electron-accepting capacity becomes stronger with lower LUMO energy. This further corroborates that hydroxylated C18:1 fatty hydroxamic acid had better interaction for the antioxidant and antimicrobial activities of ADX when compared with the other major constituents as its LUMO energy value was the least. The energy gap (ΔE) was calculated as:

$$\Delta E = E_{LUMO} - E_{HOMO}$$

The energy gap is an expression of electronic bonding. The lower the value, the better the bonding and

**Table V -** Molecular properties of ADX calculated using DFT at B3LYP/6-31G basis set level for major fatty acids in ADX

| Quantum Chemical Property | C16:0 | C18:1 | C18:2 |
|---|---|---|---|
| Molecular surface area (Å$^2$) | 377.47 | 419.86 | 436.05 |
| Energy (au) | -824.65 | -1051.16 | -1200.04 |
| $E_{HOMO}$ (eV) | -10.83 | -10.87 | -10.70 |
| $E_{LUMO}$ (eV) | 5.02 | 4.63 | 4.65 |
| $E_{LUMO-HOMO}$ (eV) | 15.85 | 15.50 | 15.35 |
| Dipole moment (debye) | 3.33 | 6.17 | 8.27 |
| Solvation (kJ/mol) | -14.45 | -22.56 | -44.11 |
| Volume (Å$^3$) | 330.46 | 380.15 | 394.63 |
| Polarizability | 64.47 | 68.59 | 69.79 |
| η (eV) | 7.93 | 7.75 | 7.68 |

hence the antioxidant and antimicrobial properties of ADX. The surface area may be related to reactivity in terms of site availability, but this may not actually connote functionality since functionality is dependent on the number of active sites available on the ADX surface. Among those considered as major constituents of ADX, hydroxylated C18:2 fatty hydroxamic acid had the highest surface area followed by hydroxylated C18:1 fatty hydroxamic acid and C16:0 fatty hydroxamic acid. The dipole moment reflects reactivity. The higher the value, the better the process is achieved. Both hydroxylated C18:1 fatty hydroxamic acid and hydroxylated C18:2 fatty hydroxamic acid had higher values than C16:0 fatty hydroxamic acid that may be due to the presence of the hydroxyl functional groups in hydroxylated C18:1 fatty hydroxamic acid and hydroxylated C18:2 fatty hydroxamic acid. The solvation energy also increases as the hydroxyl functional groups decrease with hydroxylated C18:2 fatty hydroxamic acid having the least value of -44.11 kJ/mol. Absolute hardness (η) is an important parameter that was taken into consideration; it measures the reactivity and stability of ADX. η was calculated as:

$$\eta = \frac{E_{LUMO} - E_{HOMO}}{2}$$

The antioxidant and antimicrobial properties of ADX must have taken place at regions with molecules having greatest softness and lowest hardness. Molecules, which are hard, have a large energy gap while soft molecule ones have a lower energy gap, which makes soft molecules more readily available for the exhibited properties than hard molecules.



## CONCLUSION

Oil has been extracted from AD and used as the starting material for the production of ADX. The fatty acid composition of AD was determined while ADX was screened for in vitro antimicrobial and antioxidant activities. The result revealed the oil yield of AD to be 19.64% while the most abundant fatty acid was C18:1 (36.55%). From the biological assays, it may be concluded that ADX possesses functional biological values, that could alleviate the effect of some drug resistant pathogenic organisms. The quantum chemical analysis showed that both LUMO and HOMO are distributed over the molecules of ADX as a result of the presence of lone pairs of electrons from nitrogen and oxygen in its molecule that promoted its activities as an antioxidant and antimicrobial agent, an activity that occurred via donor-acceptor interactions.

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