ISSN: 1119-1104

ISOLATION AND CHARACTERIZATION OF 9,12,15-OCTADECATRIENOIC ACID FROM THE DE-FATTENED SEEDS OF *CHENOPODIUM AMBROSIOIDES* LINN

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Received: Apr., 2018; **Accepted:** Aug., 2018

ABSTRACT

The de-fattened seed powder of *Chenopodium ambrosioides* Linn was extracted twice using n-hexane after which the urea and thiourea adduction was carried out using saturated urea in acetone and saturated thiourea in methanol respectively. Gas chromatography- mass spectrometry (GC-MS) analysis was performed on adducts. The GC-MS analysis showed that the urea adducts were straight chain compounds, while the thiourea adducts were branched and cyclic compounds.The compound, 9,12,15-Octadecatrienoic acid was among the compounds detected from the seeds of *C. ambrosioides* Linn.for the first time. The adducts and non-adducts obtained was also tested for antimicrobial activity on *Escherichia coli, Staphylococcus aureus, Candida albicans* and *Salmonella SP*. There was activity on *Staphylococcus aureus* while a slight activity was observed in *Candida albicans* but no activity on *Salmonella SP* and *Escherichia coli*.thiourea non-adduct showed more activity.

Key words:*Chenopodium amnbrosioides*, Urea, thiourea adduction, antimicrobial.

INTRODUCTION

Pain and ailments have always been one of the causes of high mortality in man and animal. It is for this reason that man has always relied on natural products for most of his cures. Natural product chemistry plays an important role in health care system. The World Health Organization (WHO) estimates that approximately 80% of the world's population relies on natural sources for primary medical treatment. The remaining 20% also incorporate natural sources in their medical treatment (WHO, 2003). Natural product chemistry can be defined as the exploration of nature in search of novel drugs or drug leads (Newmann *et al.*, 2000; Cragg, 2002). Of the about 2,500,000 species of plants, it is estimated that only 5-15% have been investigated for natural products (Cragg *et al.*, 2001). Therefore, natural product chemistry has a wider scope to explore natural resources in search of natural products.

Plants have formed the basis of sophisticated traditional medicinal systems that have been in existence for thousands of years, and continue to provide humanity with new remedies. The importance of plants in human and animal health is evident in the increasing presence of natural product drugs in modern medicine. Indeed, natural products and their derivatives represent more than 50% of all the drugs in clinical use in the world today (Gurib-fakim, 2006).

The current advancement in science has made it possible for the isolation of compounds of medical importance from plants that are used in traditional practices. Different parts of plants such as leaves, roots, stem barks, seeds and rhizomes are often extracted using different solvents. A scientific evaluation of medicinal plants according to their traditional claims could be incorporated into the complementary and alternative medical system. One such plant is *Chenopodium ambrosioides* Linn. (Family:*Chenopodiacaea*) which is being used in the present investigation.

In traditional medicine, seeds and fruits of *Chenopodiumambrosioides* are used as anthelmintic chiefly for ascaris and for intestinal amoebae. Decoction prepared from its leaves and flowers is used as remedy for fibroids and uterine haemorrhage. The essential oil of *C. ambrosioides* has been reported to possess antiulcer and antiprotozoal activities in mice (Sundaram *et al.*, 2010). The flavonoids and terpenoid compounds isolated from the plant have antioxidant and anticancer effects. In Homoeopathy, C*henopodium ambrosioides* has been prescribed for the treatment of worm infestation, while the methanol extract of its leaves has been reported to possess anti-inflammatory effect (Lohdip and Aguiyi, 2013).

The aim of this present work is to isolate 9,12,15-octadecatrienoic acid from the seeds of *Chenopodium ambrosioides* by urea-thiourea adduction and characterize by spectral analysis, as well as to test for possible antimicrobial activities of the adducts/non-adducts.

The outcome of this research would be reporting the presence of 9, 12,15-octadecatrienoic acid in the seeds of *Chenopodium ambrosioides* Linn for the first time. It is also expected that this research would be able to present the seeds of *Chenopodium ambrosioides* as a starting point for possible drug/food supplement development.

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MATERIALS AND METHODS

Sample Collection and Preparation

The plant *Chenopodium ambrosioides* Linn was collected during the rainy season and was authenticated and deposited at the Department of biological sciences, Ahmadu Bello University, Zaria, and collaborated at the Herbarium, Department of Horticulture, Federal College of Forestry, Jos, with Voucher number 1921.

The seeds from the plant were obtained by uprooting the fresh plant and dried at room temperature. The seeds were removed from the plant, cleaned with distilled water to remove the dirt and then air dried at room temperature to remove moisture. The dried seeds were then ground with mortar and pestle and the powder stored in clean polyethene bags. The pulverized sample was then de-fattened with n-hexane and the de-fattened sample dried at room temperature.

Extraction

The powdered seed (500 g) was defattened with n-hexane by soxhlet extraction method and the residue air dried. The extract obtained here was concentrated *in vacuo* and dried; this was taken as the fat and calculated as oil content. The dried residue was then extracted by maceration with n-hexane for four days (Twice) and then filtered. The n-hexane extract was concentrated *in vacuo* to obtain the crude extract. The percentage yield of the crude extract was determined and the crude extract stored in a refrigerator for future use.

Urea and Thiorea Adduction

The method used was that employed by (Akpuaka et al., 2013).

Urea adduction: The dried extract (0.3g) was dissolved in n-hexane. A saturated solution of urea in acetone was then added gently to the dissolved saturated extract in n-hexane. A white crystalline precipitate was obtained on addition until no more crystals were formed with the addition of more saturated urea in acetone solution. The crystals were then filtered off, washed with n-hexane and then dissolved in distilled water. The dissolved crystal in distilled water was then extracted several times with n-hexane in a seperating funnel. The straight chain compounds were extracted in n-hexane which was then air dried in a clean pre- weighed vial to a constant weight.

Thiourea adduction: The filtrate of the urea adduction, ie. the non- adduct, was air- dried and dissolved in benzene. Saturated solution of Thiourea in methanol was prepared. Urea non- adduct in benzene was mixed with the Thiourea solution in methanol in a ratio 1:1 and left at room temperature for four days for crystal formation. The crystalline material formed was filtered off, washed several times with benzene, allowed to dry and then dissolved in distilled water. The dissolved crystal was extracted with n-hexane several times using a seperating funnel and was evaporated to dryness in a pre- weighed clean dried vial and weighed again (Selecky *et al*., 1978). The Thiourea non- adduct (the filtrate) was evaporated to dryness in a Pre-weighed dry vial and weighed again.

Fourier transform- infrared (FT-IR) and gas chromatography- mass spectroscopy (GC-MS) analyses were performed on the thiourea non-adduct.

Antimicrobial Bioassay:

The thiourea non-adduct was tested for antimicrobial activity against *Escherichia coli*, *Candida albicans*, *Staphylococcus aureus* and *Salmonella* SP were maintained in nutrient agar medium. Antimicrobial assay was done by agar well diffusion method (Washington H.A, 1985) on nutrient agar medium using 100 μL of thiourea non-adducts against test organisms (107cfu/ml). Antimicrobial capability was estimated visually by measuring the inhibition zone in mm.

RESULTS

Percentage Yield and Moisture Content

Percentage yield of crude n-hexane extract obtained from the defatted seeds, as well as oil and moisture contents of the seed powder are shown on Table 1.

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Table 1: Percentage Yield of Crude n-Hexane Extract Obtained from the Defatted Seeds, Oil and Moisture Content of the Seed Powder of *C. ambrosioides*

Parameter	**Value**
Weight of sample powder	500 g
Weight of crude extract	5.65 g
Percentage yield	1.13 %
Moisture content	6.32 %
Oil content	2.58 %

Fourier Transform- Infrared (FT-IR) Analysis

The peaks of Fourier transform- infrared (FT-IR) of the thiourea non-adducts are shown in Figure 1.

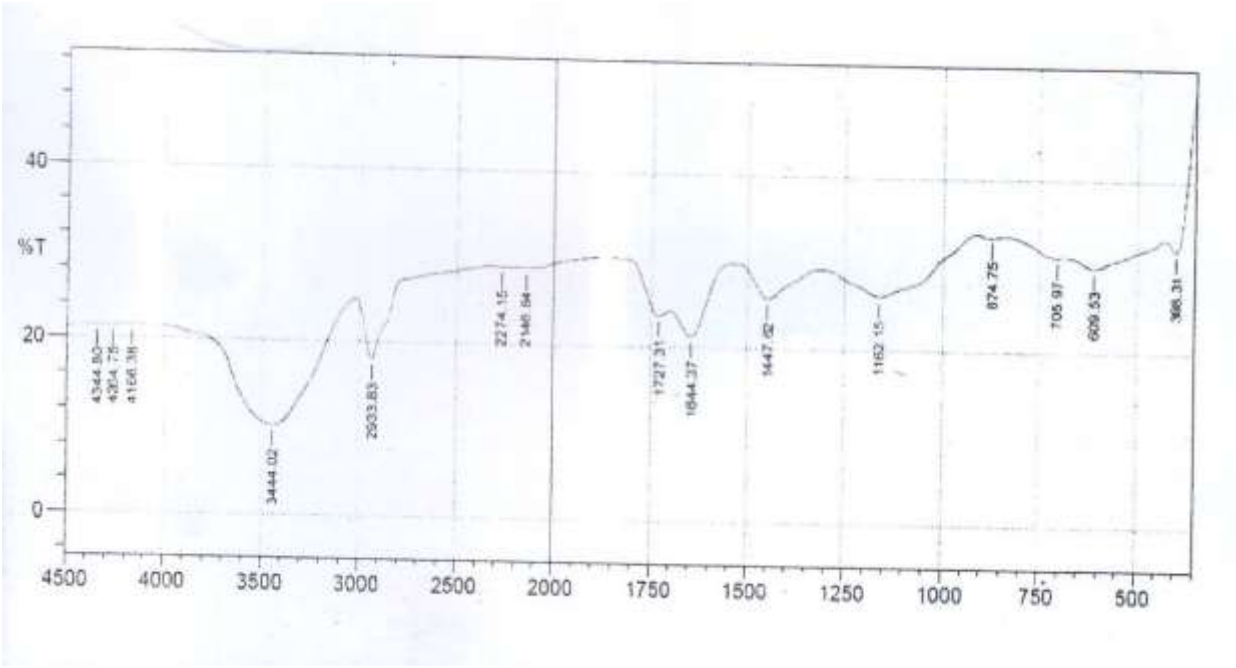

Figure 1: FT-IR Spectrum of the Thiourea Non-adducts of *C. ambrosioides*

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The FT-IR Spectrum of the thiourea non-adducts of *C. ambrosioides* L. shows the O-H(str) peak at 3444.02 cm^{-1}, C-O(bend.) at 1162.15 cm^{-1} and O– H(bend) for carboxylic acid at 1447.62 cm^{-1}, C = O(str) peak for unsaturated acids at 1727.31 cm^{-1}, alkenyl C = C(str) for unconjugated olefins at 1644.37 $cm^{-1}$and alkenyl C –H peaks at 2933.83 cm^{-1} and 705.97 cm^{-1}.

(*https://wwww.orgchemboulder.com/spectroscopy/...; https://wbspectra,chem.ucla.edu/irtable.html*).

All these support the fact that the thiourea non-adducts is a carboxylic acid.

Gas Chromatography- Mass Spectroscopy (GC-MS) Analysis

The mass spectrum and fragrnentation patern of the urea non-adduct from *C. ambrosioides* L. are shown in Figure 2 (compared to that of 9,12,15-Octadecatrienoic acid, Figure 3) and Scheme 1.

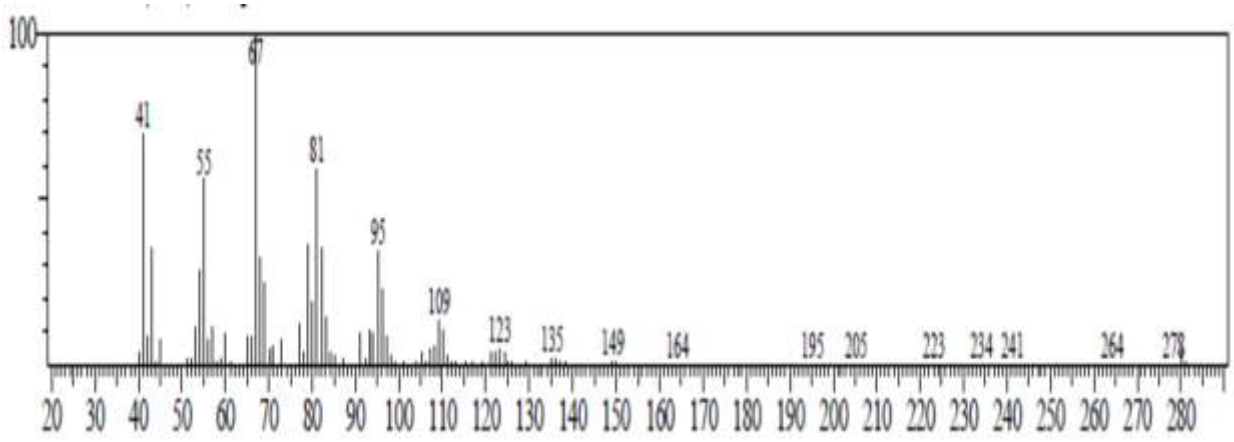

Figure 2: Maass Spectrum of the Thiourea Non-adduct of *C. ambrosioides* (9,12,15-Octadecatrienoic acid). Retention time =21.617

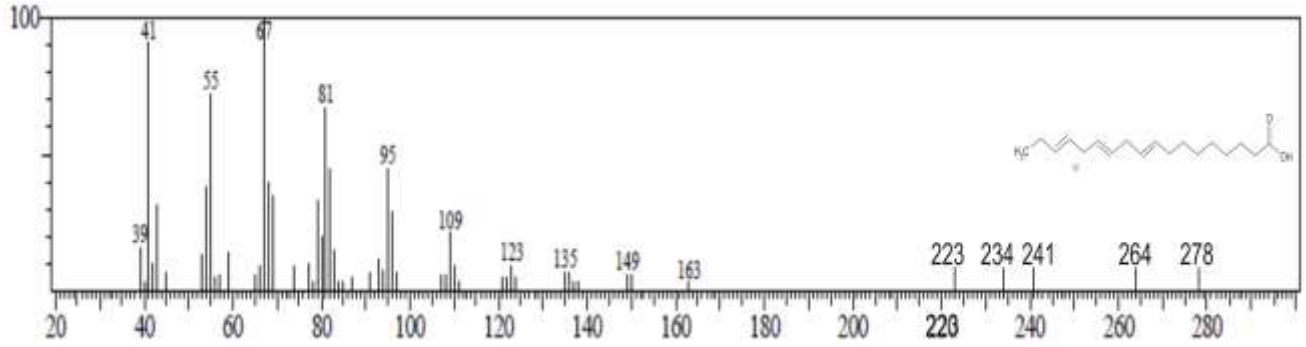

Figure 3: Mass Spectrum of 9,12,15-Octadecatrienoic Acid ($C_{18}H_{30}O_2$)

Antimicrobial Activity

Results for the antimicrobial activity of urea and thiourea adducts, as well as thiourea non-adducts are summarized on Table 2.

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Table 2: Diameter of Zone of Inhibition (mm) of Urea Adducts, Thiourea Adducts and Non-adducts of the Seeds of C. Ambrosiodes against the Test Organisms

Adduct/Non-adduct	*Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*	*Salmonella SP*
Urea	-	8	-	6
Thiourea	-	10	-	9
Thiourea non-adducts	-	19	-	13

DISCUSSION

The moisture content of the seed powder was found to be 6.32 % (Abdullahi et al., 2015), which is within the acceptable limits of about 6 to 15% for most vegetable drugs. Low moisture content reduces errors in the estimation of the actual weight of drug material, reduces components hydrolysis by reducing the activities of hydrolytic enzymes which may destroy the active components, and also reduces the proliferation of microbial colonies and therefore minimize the chance of spoilage due to microbial attack (Lohdip, 2011). The gas chromatogram showed the various peaks out of which one was identified by comparison of the mass spectrum to the data library as 9,12,15-Octadecatrienoic acid, $C_{18}H_{30}O_2$, (Figure 4).

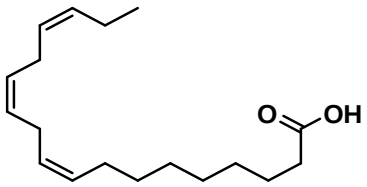
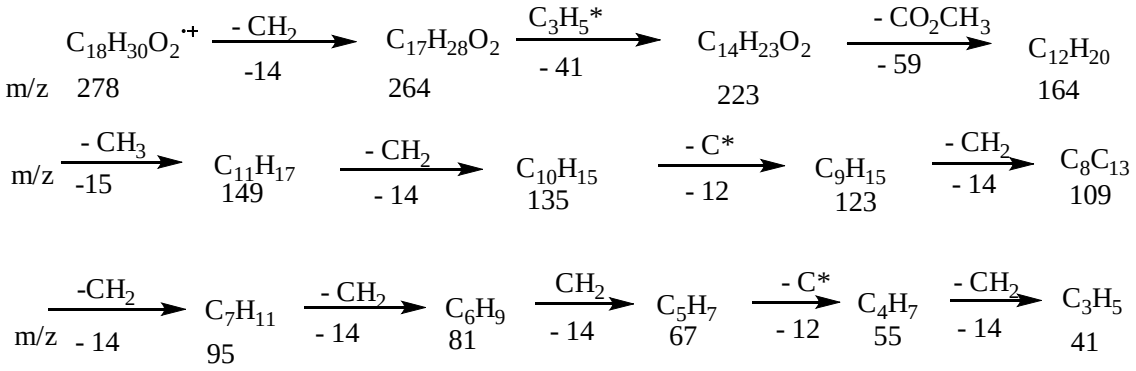


Figure 4: 9,12,15-Octadecatrienoic acid (Z,Z,Z)

The fragmentation pattern of is as shown in Scheme 1 below:



Scheme 1: Fragmentation of the Fraction from the Seeds of *C. ambrosioides* Linn

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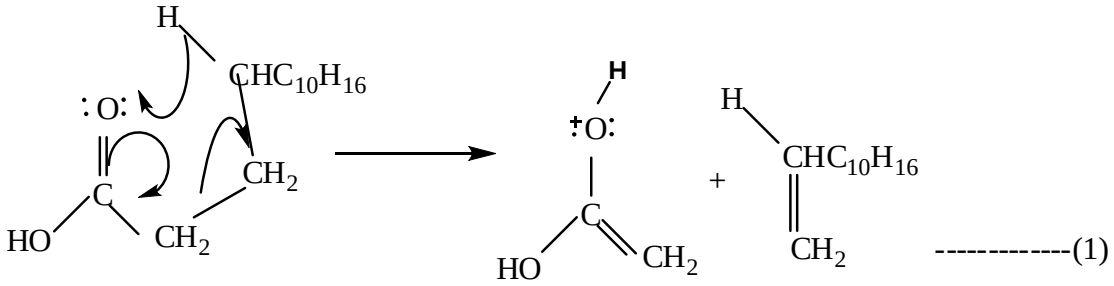
The molecular ion peak is caused by the $[C_{17}CO_2H]^{\cdot+}$ ion and corresponds to m/z 278. The fragments are odd as expected.Compounds like alcohols, nitrogen-containing compounds, carboxylic acids, esters, and highly branched compounds may completely lack a visible molecular ion. This is because even molecular ion usually produces odd molecular fragments by breaking single bonds (http://www.whitman.edu/chemistry/edsolns..).

Just like expected for carboxylic acids, the molecular ion is of low abundance; infact, compounds like alcohols, nitrogen containing organics, carboxylic acids, esters, and highly branched compounds may completely lack a visible molecular ion. The suggested molecular formula agrees with the degree of unsaturation according to the follwing formular:

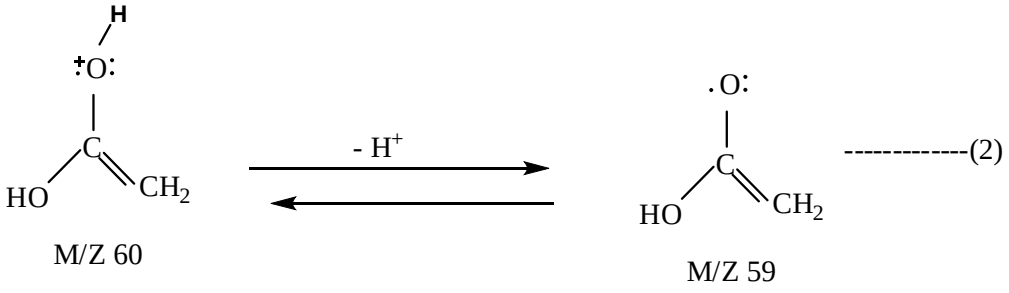
$$\text{Degree of Unsaturatiom} = C - \frac{\text{No. of H}}{2} - \frac{\text{No. of X}}{2} + \frac{\text{No. of N}}{2} + 1$$

Where X = (F, Cl,Br, and I), and N is the number of nitrogen atoms in the chemical formula. As the equation indicates, the number of oxygen atoms does not affect the degree of unsaturation (http://www.whitman.edu/chemistry/edsolns..). By using this equation the molecular formula $C_{18}H_{30}O_2$ gives four as the degrees of unsaturation. Short chain carboxylic acids give prevalent peaks at M – OH and M – CO2H. However, in larger carboxylic acids these peaks are less prevalent. Long chain carboxylic acids are better identified by the fragments at $CnH_{2}n\text{-}_1O_2$ (http://www.whitman.edu/chemistry/edsolns..; Silverstein et al., 1991). However, in the case of 9,12,15-Octadecatrienoic acid as seen in its mass spectrum (Figure 3), it does not strictly give $CnH_{2}n\text{-}_1O_2$, probably because of the illogical fragmentations as evidenced btween fragments m/z 264* and 234*. The loss of fragments from the 19-25 range is also unlikely except in the case of fluorinated compounds (http://www.whitman.edu/chemistry/edsolns..). The McLafferty rearrangement appeared, expected for carboxylic acids with a γ-H ((http://www.whitman.edu/chemistry/edsolns..) to have taken place at fragment m/z 223 ($C_{14}H_{23}O_2^{\cdot+}$):



: M/Z 223 M/Z 60 M/Z 164

However, there is difference of 1 unit, each, in the two fractions above, compared to the fragmentation of the spectrum of 9,12,15-Octadecatrienoic acid (Figure 3), which could be in the course of the rearrangement, one of the H^+ of the OH of m/z 60 remained with M/Z 164 and the former was lost as radical, since it could not appear in the spectrum.



M/Z 60 M/Z 59

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CH_3CO_2·is one of the fragments that can be lost as a radical in the course of fragmentation (Finar, 1980). Also, in long chain acids the spectrum consists of two series of peaks resulting from cleavage at the C – C bond with retention of charge either on the O-containing fragment (m/z 59 as would have been in this case) or on the alkyl fragment (in this case m/z 164) (Silverstein, 1991); this could also be the reason for the fragment m/z 164 and none for m/z 59. The fragmentations with the loss of m/z 14 for CH_2 is typical of a long-chain carboxylic acid just as in hydrocarbon (http://www.whitman.edu/chemistry/edsolns..; Silverstein et al., 1991).

The compound, 9,12,15-Octadecatrienoic acid, which is an omega 3 fatty acid, and is useful as an anti-inflammatory, antidiabetic, treats eczema, hypocholesterolemic, nematicide, pesticide, anticancer, anti-inflammatory, viral and bacterial diseases, antihistamic and antioxidant as reported by Rajagopal *et al.,* (2014) and Hema *et al.,* (2011).

The isolation of 9,12,15-Octadecatrienoic acid was made possible because of the successive n-hexane extraction that was employed. This goes a long way to prove that successive hexane extractions of plants, yields more phyto constituents than a single extraction.

The antimicrobial bioassay carried out after adduction showed a strong activity on *Staphylococcus aureus* and a slight activity on *candida albicans*. There was no activity on *Escherichia coli* and *Salmonella* SP, thoughactivity of *Candida* is low unlike that of *staphylococcus aureus*. Any extract with zone of inhibition equal to or more than 6 mm is considered active (Boda,1997; Lohdip et al., 2017). The observed antibacterial properties of the adducts and non-adducts shows the potential of the plant to cure bacterial infections (Pandey *et al.,* 2011).

From the research work carried out so far on *Chenopodium ambrosioides* Linn, this is the first time that 9,12,15- Octadecatrienoic acid is detected. These are the acids known as alpha linoleic acid (ALA) or omega-3 fatty acids. α-Linolenic acid, an *n*−3 fatty acid, is a member of the group of essential fatty acids (EFAs), so called because they cannot be produced within the body and must be acquired through diet. Most seeds and seed oils are much richer in an *n*−6 fatty acid, linoleic acid, but very few have been found to contain the n-3 fatty acids. They have very good Pharmacological importance such as anticancer, Hypocholesterolemic, antimicrobial, antioxidant and antiinflammatory activities.

CONCLUSION

The urea and thiourea adductions and the non-adducts yielded straight chain, branched and cyclic bioactive compounds respectively as reported by Ekwenchi and Mailabari (1983). These compounds have reported pharmacological use. GC-MS analysis of urea and thiourea also identified a variety of bioactive compounds, one of which is 9,12,15-octadecanoic acid (Z,Z,Z), in the urea and thiourea adduction, which could be used for the treatment of, among others, inflammatory, tumor, diabetic, heart, viral and bacterial diseases. The well known bioactivities of the isolated compounds may explain the traditional medicinal uses of the plant. Hence, the present study was justified on its use in the traditional folk medicine.

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