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Isolation and Characterization of Secondary Metabolite from the leaves *Aspilia Africana* (Pers.) C. D. Adams (Asteraceae)

Ahamefula A. Ahuchaogu* and Okenwa U. Igwe
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Abstract *Aspilia africana is a medicinal plant used traditionally for treating various diseases. Phytochemical investigation of the methanol extract of the leaves ~~has~~ led to the isolation of squalene (hexamethyl-2, 6, 10, 14, 18, 22-tetracosahexa-ene) from the leaves of A. Africana and is known to possess significant antimicrobial activity, it has not been known to be previously isolated from Aspilia Africana. The structure of the component was elucidated based on the NMR and MS techniques and by comparison of their experimental data with literature.The purified compound was obtained by chromatographic method*

Key Words: *Squalene, isolation and characterization, Aspilia Africana, MS and NMR*

Ahamefula A. Ahuchaogu*
Department of Pure and Industry Chemistry,
Abia state University, Uturu. Abia State, Nigeria
Email: ahuchaogu.aa@abiastateuniversity.edu.ng
Orcid id:0000-0002-6412-7487

Okenwa U. Igwe
Department of Chemistry,
Michael Okpara university of Agriculture,
Umudike, Abia State, Nigeria
Email: okenwauigwe@gmail.com

1.0 Introduction

Plants have immense potential that can be explored for the management and treatment of various human ailments, green synthesis, production of industrial chemicals and other applications (Thakur *et al*., 2011; Rekik *et al*.,2019;) Indeed, several medicinal plants have been used in traditional medicine for the treatment and management of all kinds of disease across the globe since time immemorial (Imran *et al*., 2015; Sabale *et al*.,2012; Agyare *et al*.,2016). In South-eastern Nigeria, some indigenous plants are used in African traditional medicine to cure diseases and heal injuries. *Aspilia africana* is one of such medicinal plants, which are fast gaining recognition due to its ability to stop blood flow in fresh wounds. *Aspilia africana* (Pers.) C. D. Adams (Asteraceae), commonly referred to as wild sunflower, is one of the highly valued
wound healing plants throughout its distribution range and beyond (Komakech *et al*.,2019). This unique wound healing plant species is commonly referred to as "hemorrhage plant" due to its distinguished ability to stop bleeding on fresh wounds, even of severed artery (Okoli *et al.,* 2007; Ukwueze *et al*.,2013; Dimo *et al.,*2002). Apart from its enormous potential in wound healing, *A. africana* is reported to be vital in the treatment and management of myriad of other diseases and disorders in African traditional medicine, including headache, corneal opacities, stomach disorders, cough, gonorrhea, rheumatic pains, and tuberculosis; the leaf infusion is taken as a tonic for women immediately after delivery (Oyesola *et al*.,2010; Okwuonu *et al*.,2017) . *A. africana* plant is also reported to possess great anti-inflammatory, antimalarial, and antimicrobial activities (Okoli *et al*.,2007; Ukwueze *et al*.,2013). Several scientific studies have attributed the numerous medicinal properties of *A. Africana* to the presence of bioactive secondary metabolites in different parts of the palnt including alkaloids, saponins, tannins, glycosides, flavonoids, and terpenoids (Okwuonu *et al*.,2017 ; Etiosa and Chika.,2018). Hanna and Niemetz (1987) reported that *Aspillia africana* has an anticoagulant activity, Okwu and Josiah (2006) also stated that infusion of the plant leaf in combination with clay has been traditionally accepted as a medicine for stomach trouble. Other therapeutic applications of the plant is in treatment of malaria infection (Okokon *et al*.,2006).
In African traditional medicine, *Aspilia africana* has been reported as an effective medicinal plant due to its wide therapeutic applications (Akujobi, *et al*.,2004). Noted that several parts of the plant; root, leaf, stem or whole plant can be used for herbal remedy either in dried form or as crude extracts. *Aspilia africana* is one of the plants that have been reported to contain a wide range of biological activities such as: antiviral, fungicidal and anti-

bacterial activities due to the presence of secondary metabolites especially Thiarubrine A (Masato and Wu., 1994). In Kenya, it is used in eliminating intestinal worms from the gastrointestinal tracts. In Uganda, it is used in the treatment of gonorrhea (Page *et al*.,1992). In Ghana, the root decoction is taken for tuberculosis while the leaves are use as cough medicine for children. Decoctions from the leaves are used to treat eye problems and serve as lotion for the face to relieve febrile headache. They are used to treat ringworm and dysentery (Abii, and Onuoha, 2011). In Cameroon ethno medicine, it is used in the treatment of stomach ailments. In Nigeria, the leaves of *Aspilia africana* are used in wound healing, treatment of skin diseases and eye infections (Okoli *et al*., 2007).

In spite of established medicinal and pharmaceutical applications of this plant, little is known of the actual phytochemicals that are present in the plant and which of them are effective against the respective purpose. A search towards the generation and documentation of information needed to established these phytochemicals and their respective roles in pharmaceutical or medicinal application requires a comprehensive analysis of the plants. Therefore, the present study is aimed at identifying the secondary metabolites in this plant using ^{1}HNMR and ^{13}CNMR spectroscopic method.

2.0 Materials and Methods

2.1 Sample collection

The leaves of *Aspilla africana* were harvested from a botanical garden in the National Root Crop Research Institute, Umudike Abia State, Nigeria on the 14th October, 2019. Authentication of plant materials was done by Dr. O. Emmanuel at the Department of Plant Science and Biotechnology, Abia State University, Uturu, Nigeria. Voucher specimen [ABSU/ AA/1778] was allocated at the herbarium in the the Botanical Department of Biological Sciences School of the same University

2.2 Preparation of plant Leaves

Fresh plant leaves were washed with running tap water for 5 minutes to remove the dust and debris and rinsed with sterile distilled water. The sample was air dried on the laboratory bench for fifteen days at room temperature to avoid decomposition of thermo labile compounds. The dried sample was milled to coarse powder using an electric blender. 300 g of the pulverized leaf material was mixed with 500 ml of solvent (95 % methanol) and introduced into a rotary shaker operating at 100 rpm for twenty-four hours. The resulting solution was filtered using a Whatman No.1 filter paper. The extract was concentrated under reduced pressure using Digital Heidolph Rotary evaporator (4000 series) and the supernatant plant extract was decanted after complete removal of the solvent. The extract was suspended in water and was successively partitioned using petroleum ether/ chloroform, ethyl acetate and methanol to obtain petroleum ether, chloroform, ethyl acetate and methanol soluble fractions, the chloroform soluble fraction was used for this study.

2.3 Isolation and purification of the compounds

The column used was 280 mm in height and 35 mm in diameter. The column was washed and rinsed with solvents of different polarities and finally with the solvent to be used. After drying, small cotton wool was used to cover the base of the column for the packing will rest on, so that the silica gel does not wash out of the bottom of the column. 400 g of the silica gel (200 meshes) was mixed with petroleum ether in a beaker. The gel was poured into the column and tapped to remove any trace of air bubbles. 5.2 g of chloroform fraction was use for silica gel column chromatography. The column was eluted with 100% petroleum ether, petroleum ether -chloroform mixture (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35,60:40, 55: 45, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95) successively chloroform-ethyl acetate solvent mixture; (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35,60:40, 55: 45, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95) then followed by ethyl acetate-methanol solvent mixture (95:5, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35,60:40, 55: 45, 40:60, 35:65, 30:70, 25:75, 20:80, 15:85, 10:90, 5:95,100) collections was made on (50 ml each). A total of 36 eluents were collected based on their TLC profiles, some of the fractions with the same R_f values were pooled together (Haruna *et al*.,2017).

2.4 Spectroscopic analysis

The spectroscopic analyses of the isolated compound were recorded on a Bruker AVANCE-600 (500MHz) for ^{1}HNMR and 150 MHz for ^{13}CNMR in deuterated chloroform using tetramethyl silane (TMS) as an internal standard. Fig. 1 shows the structure of the isolated compound AA-1.

4.0 Results and Discussion

2.8 mg of Compound AA-1 was isolated using column chromatography as a brownish yellow gum.The column chromatography isolation was done with gradient elution using different mobile phases, the fraction of chloroform / ethyl acetate (55:45) with R_f 0.79 derived from the chloroform fraction of the leaf extract of *Aspilla Africana* and identified as ~~known~~ squalene which has been isolated from *Hemigraphis hirta* (Khurshid.,2002). Structural elucidation of AA-1 (Fig.1) was principally achieved using NMR and MS and comparison with literature as shown in Table 1 and 2 .

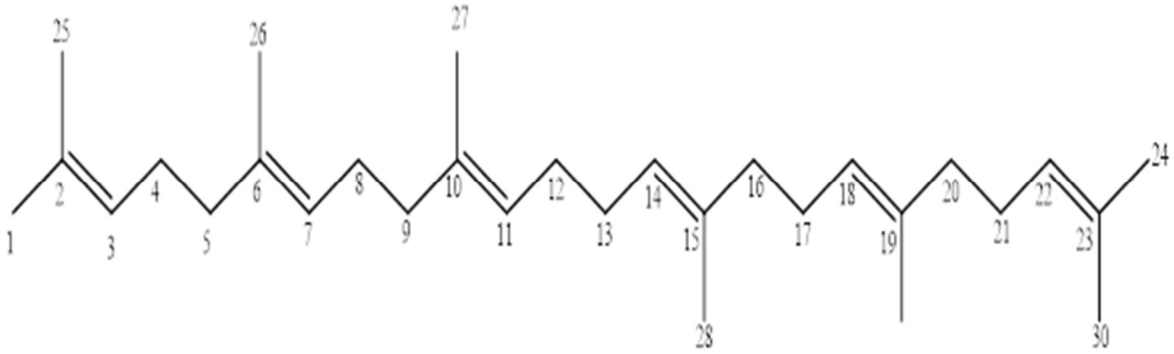

Fig 1; The structure for compound AA1: Squalene (Hexamethyl-2, 6, 10, 14, 18, 22-tetracosahexa-ene)

Table 1: ^{1}HNMR data for compound AA-1: Squalene

No	13C NMR (150 MHz) δ_C ppm	13C NMR Lit (100 MHz) δ_C ppm	Nature of carbon
1/24	25.8 CH3	25.7 CH3	Primary
2/23	131.1C	131.2 C	Quaternary
3/22	124.3 CH	124.2 CH	Tertiary
4/21	26.7 CH2	26.8 CH2	Secondary
5/20	39.7 CH2	39.8 CH2	Secondary
6/19	134.9 C	134.8 C	Quaternary
7/18	124.3CH	124.4 CH	Tertiary
8/17	26.7 CH2	26.6 CH2	Secondary
9/16	40.0 CH2	39.7 CH2	Secondary
10/15	135.0 C	135.0 C	Quaternary
11/14	124.2 CH	124.3 CH	Tertiary
12/13	28.2 CH2	28.3 CH2	Secondary
25/30	17.7 CH3	17.6 CH3	Primary
26/29	16.0 CH3	16.0 CH3	Primary
27/28	16.0 CH3	16.0 CH3	Primary

*** Overlapped protons**

Table 2: ^{13}CNMR data for compound AA-1: Squalene

No	13C NMR (150 MHz) δc ppm	13C NMR Lit (100 MHz) δc ppm	Nature of carbon
1/24	25.8 CH3	25.7 CH3	Primary
2/23	131.1C	131.2 C	Quaternary
3/22	124.3 CH	124.2 CH	Tertiary
4/21	26.7 CH2	26.8 CH2	Secondary
5/20	39.7 CH2	39.8 CH2	Secondary
6/19	134.9 C	134.8 C	Quaternary
7/18	124.3CH	124.4 CH	Tertiary
8/17	26.7 CH2	26.6 CH2	Secondary
9/16	40.0 CH2	39.7 CH2	Secondary
10/15	135.0 C	135.0 C	Quaternary
11/14	124.2 CH	124.3 CH	Tertiary
12/13	28.2 CH2	28.3 CH2	Secondary
25/30	17.7 CH3	17.6 CH3	Primary
26/29	16.0 CH3	16.0 CH3	Primary
27/28	16.0 CH3	16.0 CH3	Primary

*** *(Source: Khurshid.,2002)**

The MS spectrum (Fig. 2) showed a molecular ion peak at m/z 410, which corresponded to a molecular formula of $C_{30}H_{50}$. The ^{1}H NMR spectrum (Fig.3) showed six olefinic proton resonances at δ_H 5.10 ppm which were assigned to H-3/22, H-7/18 and H-11/14, Eight methyl proton resonances at δ_H 1.67, 1.60, 1.60, and 1.60 assigned to H-1,24 ,H- 25,30, H- 26,29, and H-27,30 and ten methylene proton resonance at δH 2.07, 1.98, 2.07, 2.03 and 2.01 which were assigned to H-4,21, H-5,20, H-8,17, H-9,16, H-12/13.

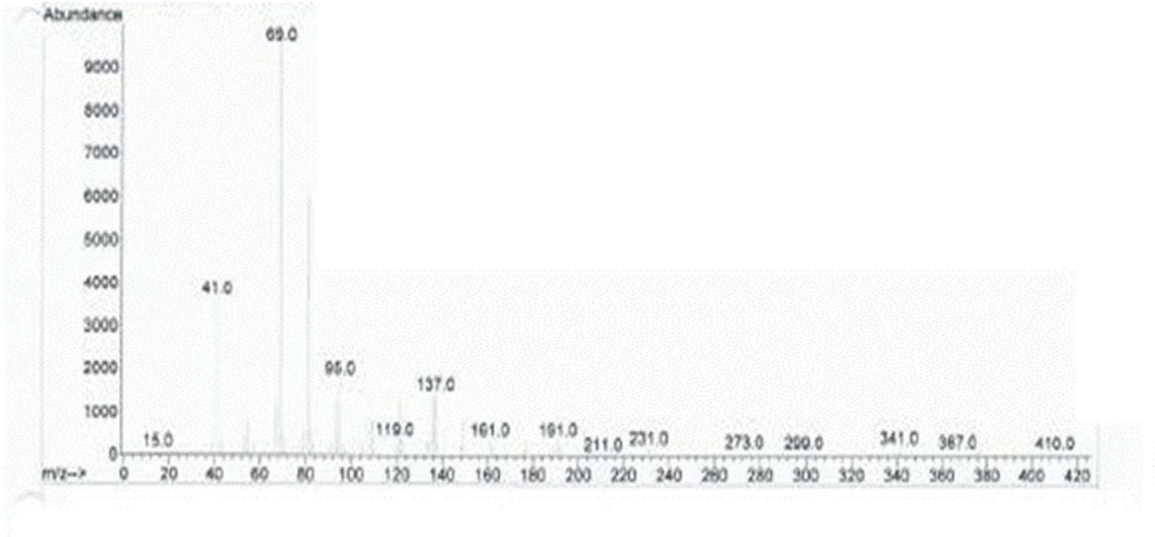

Fig. 2: Mass Spectrum of compound AA-1

The ^{13}C NMR spectrum (Fig. 4) displayed fifteen carbon resonances representing thirty carbons, including eight methyl, ten methylene, six methines and six trisubstituted carbons. The ^{1}H and ^{13}C NMR spectral features of this compound were compared with those in literature (Khurshid.,2002) and was found to be similar (Tables 1 and 2). The signals at 131.2 ppm, 134.9 ppm, 135.0 ppm 135.0 ppm, 134.9 ppm, 131.2 ppm were due to quaternary carbons. The DEPT (Fig.5) provided information on the nature of carbon present. Six methine carbon (-CH) had signals at 124.3 ppm , 124.3 3 ppm ,124.2 3 ppm ,124.2 3 ppm,124.23 ppm , 124.3 3 ppm, 124.3 3 ppm which were assigned to the following carbons C-3,C-7, C-11,C-14, C-18 ,C-22

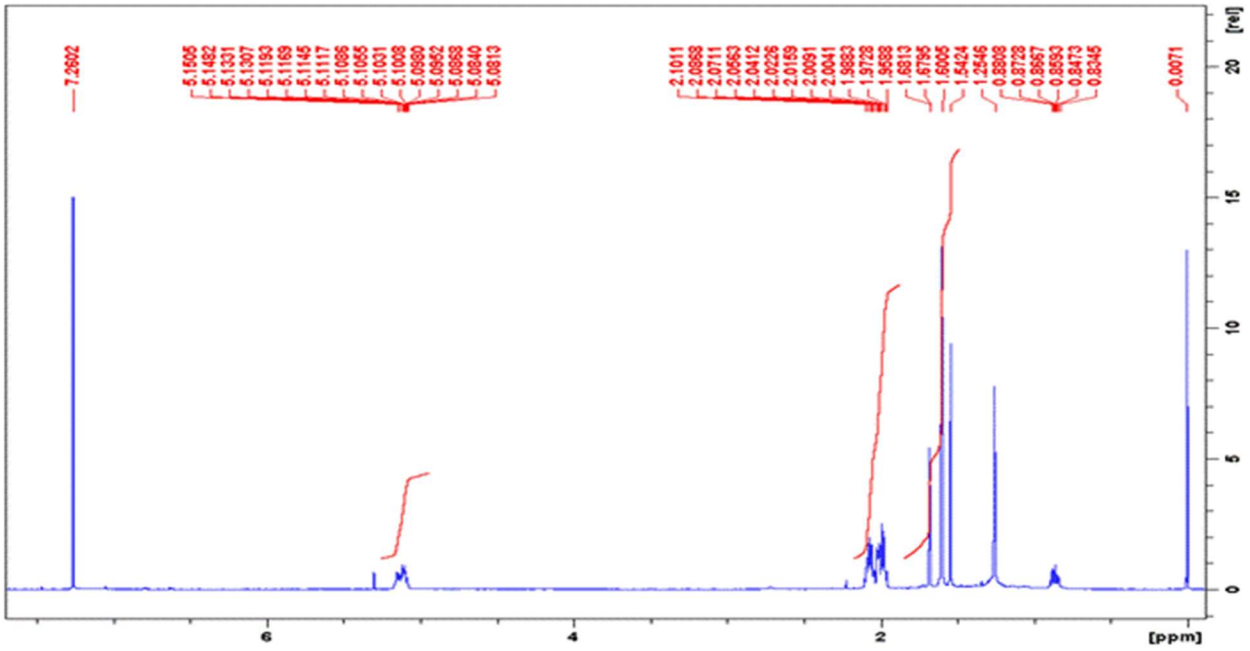

Fig 3 : ^{1}H NMR Spectrum of compound AA-1

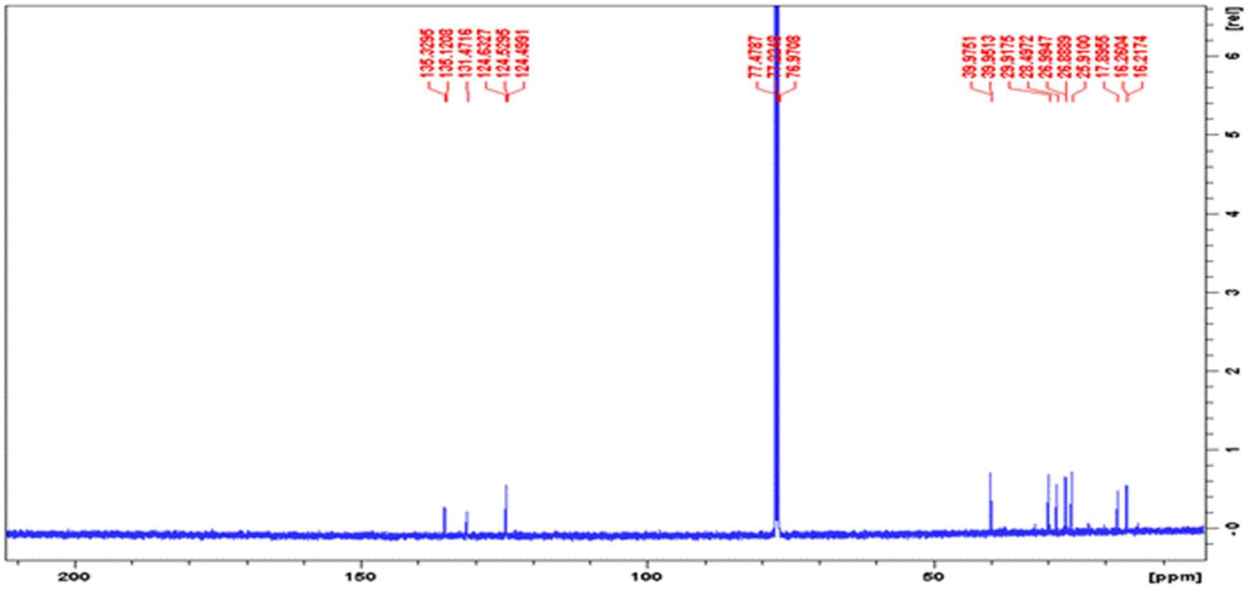

Fig 4: ^{13} C NMR Spectrum of compound AA-1

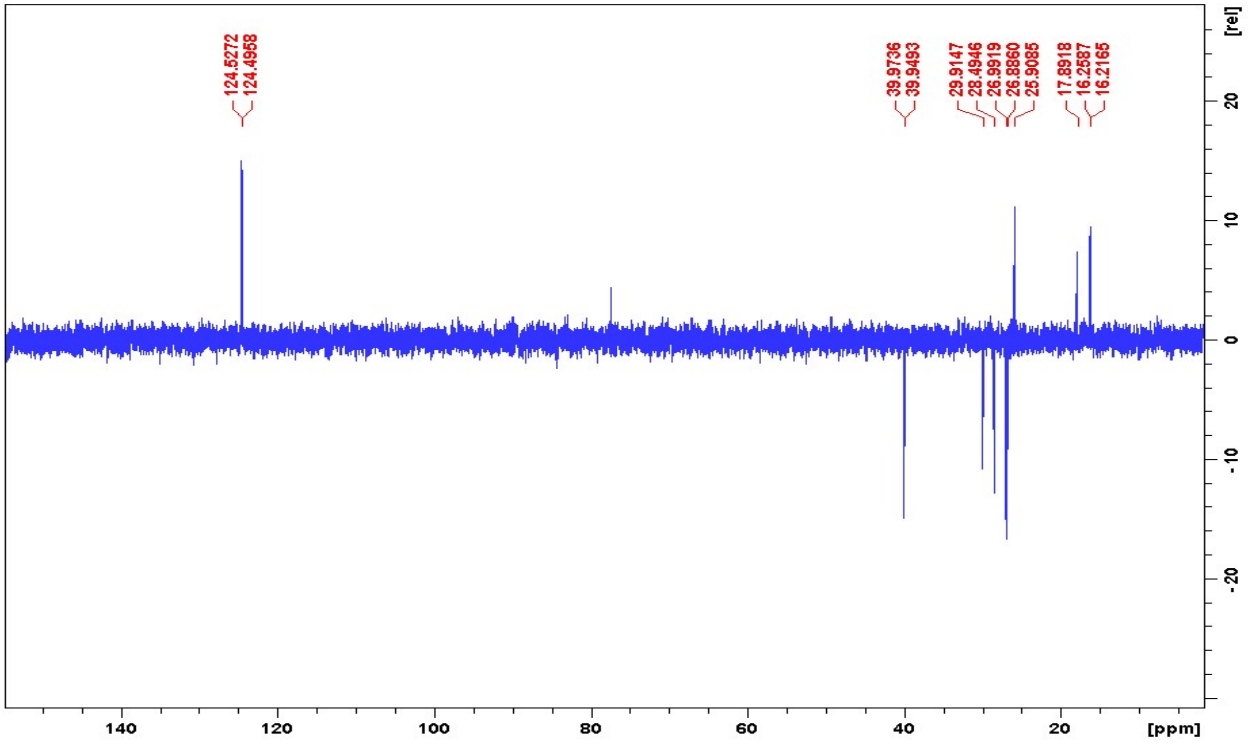

Fig 5: ^{13} CNMR DEPT Spectrum compound of AA-1

4.0 Conclusion

Extraction, isolation and purification of chloroform fraction from the leaf extract of *Aspilla africana* has led to the isolation and characterization of a triterpene (squalene) using Nuclear Magnetic resonance spectroscopy and Mass spectrometry. This is the first report of this compound from this plant.

5.0 References

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