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CHARACTERIZATION OF EUDESMENES FROM THE SEED AND ROOT EXTRACTS OF ANNONA MURICATA

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ABSTRACT

The air dried powdered seed material of *Annona muricata* was exhaustively subjected to extraction using methanol 100% and the extract was further re -submitted to Vacuum Liquid Chromatography (VLC) using silica gel and eluted with solvent of various polarity to afford 14 major fractions (F_1-F_{14}). Fraction (F-9) was further subjected to a repeated gel filtration to afford compound 1 (32 mg). The air dried powdered root material of *A. muricata* was exhaustively subjected to extraction using methanol 100%. The combined methanol extract was dissolved in water and subsequently partitioned with n-hexane, chloroform, ethyl acetatae and n-butanol. The chloroform ($CHCl_3$) soluble fraction was submitted to column chromatography over silica gel with n-hexane / EtOAC (8:0 – 0:1) as eluent to give fractions B_{1-10}. Fractions $B_{4,5,6 \text{ and } 7}$ where pooled together and was re-submitted to repeated column chromatography using sephadex LH_{20} with MeOH /$CHCl_3$ (3:1) to give compound 2 (28 mg). Their structures were elucidated mainly by using a FTIR, FABMS, high resolution 1 Dimension and 2Dimension (1D and 2D) spectroscopic and chemical analysis. Compound 1 determined as rel - 1β, 3α, 6β – trihydroxyeudesm – 4 – ene while compound 2 was determined as 4-(15) – eudesmene 1β, 5α – diol.

Keywords: Anonaceae, a*nona muricata*, root, seed, sesquiterpenes, spectral data (1D and 2D)

INTRODUCTION

The therapeutic or medicinal activity of plants usually depends on the presence of what are known as active principles and some understanding of this is necessary in any study of the actions of plants and it parts as drugs. Over the century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery [1]. The importance of the active ingredients of plants in agriculture and medicine has stimulated significant scientific interest in the biological activities of these substances. Plant natural products are involved in many aspect of human

existence. These products may be used as purified compounds or as components of complex mixtures which serves as medicines, pesticides, flavourings or as herbicides [2]. Therefore, plants may be regarded as a biosynthetic laboratory that produces or synthesizes a large variety of chemical compounds during its metabolic activities. These include compounds that are utilized by man and other animals to exert physiological effects on them [3]. Medicinal plants are hence referred to as those in which one or more of their parts contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The medicinal properties of drug plants are dependent on the presence of a variety of chemical substances known as the secondary metabolite [4]. These secondary metabolites are constituents synthesized by the plant in addition to its basic metabolites, which may be concentrated in different parts of the plant. Some of these compounds include saponins, glycosides, flavonoids, anthraquinones, alkaloids, steroid and terpenoids. Others include coumarins, tannins, gum and mucilage, volatile oil and organic acids. These chemical constituents are dominant in different drug plants given it a unique chemistry which differs from one plant to another at varying concentrations [5]. The phytochemical examination of all these constituents has been made possible and easier by the improved method of extraction, separation, isolation and characterization.

It is evident, that the plant kingdom still holds many species of plants containing substances of great medicinal value which have just been discovered or yet to be discovered [6]. Large numbers of plants are constantly being investigated for their possible pharmacological values.

Anona muricata. linn, commonly known as "soursop" or "graviola" is a tropical tree with aromatic sweet and great tasting fruit which belongs to the family of "custard – Apple" plants of *Annonaceae* family. This is a deciduous, terrestrial, erect tree of 5-8 meters in height, with an open, roundish canopy and is usually found from Central America to South America and to other tropical countries around the world [7]. The leaves are known for its several medicinal uses such as remedy for headaches, insomnia [8], cystitics, liver problems [9], diabetes and hypertension [10] and also used for remedy as an anti-inflammatory [11], antispasmodic [12], and antidysentric agent [13]. The decoction of the leaves is also known to have parasiticide, antirheumatic [14] and antineuralgic uses, while the cooked leaves, if applied topically alleviate rheumatism and abscesses [15]. Literature search has shown that no much work has been reported on seed and root portion of this plant to ascertain the constituent's metabolites

responsible for its activity in the treatment of related aforementioned ailments. This prompted the screening of this plant for its bioactive compounds.

Consequent upon this, the research was aimed at isolating, and structural elucidation of some principles, present in the root and seed extract of *A. Muricata* using some standard phytochemical and spectroscopic protocols.

EXPERIMENTAL

All melting points were determined on Gallenkamp melting point apparatus and results are uncorrected. All solvents of analytical grade were purchase from BDH chemical Ltd. Poole England. TLC analysis was carried out using cellulose (TLC) and Silica gel 60 F_{254} (TLC) plates (Merck, Darmstadt, Germany). Polyamide (Roth, England) and Sephadex LH_{20} (Fluka, Switzerland) were used for open column chromatography (CC). Optical rotations were measured on Bellingham and Stanley ADP200 polarimeter (Britain) IR spectra were recorded on a Nicolet 360 FT-IR spectrophotometer (Britain) while HRESI-MS/ FABMS were measured on a mass Autospect-ultima-TOF spectrometer (Britain). ^{1}HNMR (400 MHZ) and ^{13}CNMR spectra were recorded in CD_3Cl_3 on a brukeravance DRX- 400 and $100_{MH/Z}$ spectrometer (Germany) with Tetramethyl Silane (TMS) as an internal standard. Chemical shift values (ppm) relative to NMR in solvent $CDCl_3$ (δ_H= 7.27, δc 77.00). Coupling constants (J=values) are given in H_Z with (TMS) as internal standard. H – H COSY, HMBC and HMQC experiments were recorded with gradient enhancements using sine – shaped gradient pulses. TLC was carried out on plates precoated with RP – 18 (Merck) and Silica gel F_{254} (Qingdaomarine chemistry Ltd, (Britain). Spots on the plates were visualized by spraying with 10% H_2SO_4 followed by heating in the oven. Column chromatography was performed on Silica gel 60 (0.040 – 0.0653mm) and column (40 - 63µm) 310mm and 15mm i.d). TLC Visualization was done by UV absorption at 254nm. All solvent were redistilled before use.

PLANT MATERIAL

The whole plant material *A. muricata* was collected separately from a farm land in Basawa, Zaria, Kaduna State, Nigeria. The plant was identified and authenticated where a voucher specimen (No. 1620) was deposited at the herbarium of Biological Science, Department of Ahmadu Bello University, Zaria, Nigeria.

Extraction and isolation

The seed and the root of the plant *A. muricata* were obtained separately and air dried at room temperature for 5 days. The seed and the root were then crushed and ground into a fine powder separately. The powdered seed and root material (680 g) and (750 g) were extracted exhaustively with methanol by cold maceration technique. The combined methanol extracts with a greenish brown mass were separately reduced to dryness in vacuo using rotary evaporator to afford 25g and 32g each for the seed and the root respectively.

The seed methanol extract was dissolve in water and partitioned sequentially with n-hexane, chloroform, ethylacetate and n-butanol. Vacuum – liquid chromatography of the n-hexane portion of the methanol extract (4.0 g) was performed using a step gradient of 10% EtOAC in hexane followed by a final methanol wash to yield 10 fractions of 20 ml each (A_1 – A_{10}). Column chromatography (CC) of fraction A_6 (1.8 g, 6:4 n-hexane – EtOAC) employing an 8:2 n-hexane – EtOAC Isocratic system was employed. This was followed by repeated preparative thin layer chromatography (PTLC) 105mg, 7:3 n-hexane: EtOAC, 3 developments) to afford (32mg), which was coded as compound 1.

Theroot methanol extract (22g) was also subjected to partitioning with n – hexane, chloroform, EtOAC and n- BuOH. The various partitioned portions were concentrated using rotary evaporator glass wares to afford n – hexane (2.5g), chloroform (5g), EtOAC (2.3g) and n – Butanol 6.2g respectively. The chloroform soluble fraction (3g) was submitted to column chromatography over silica gel with n-hexane/EtOAC (8:0-0:1) as eluent to give fractions $B_{1\text{-}10}$. Similar fractions were pooled together on the basis of TLC profile. Fraction $B_{4,\,5,6}$ where further pooled together and resubmitted to repeated gel filtration using sephadex L_{20} with MeOH/$CHCl_3$ (3:1) to afford compound 2 (23g).

RESULTS AND DISCUSSION

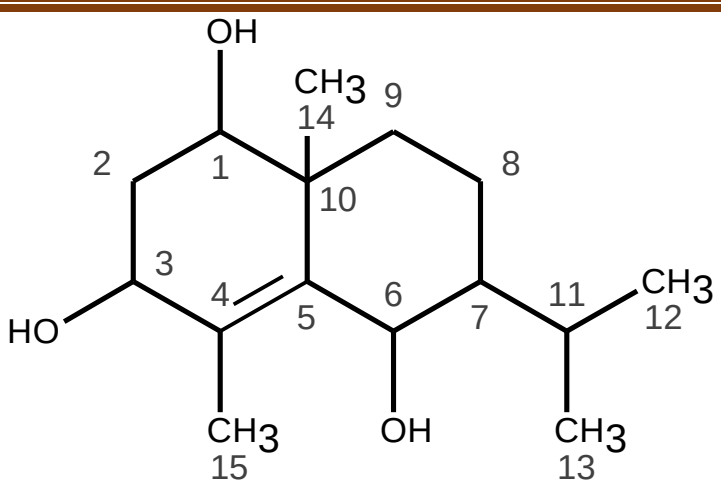
Table 1:^{1}H NMR and ^{13}C NMR Spectrum data of Compound 1in $CDCl_3$. (400.$_{MHZ}$ -^{1}H and 100$_{MHZ}$for^{13}CNMR). Multiplicity and Coupling constant (J, Hz) δ ppm.

Position	^{1}H	CH	^{13}C
1	3.48(12.4)	CH	74.5
2	1.76m	CH_2	32.3
3	4.36bs	CH	84.5
4	-	C	125.6
5	-	C	145.3

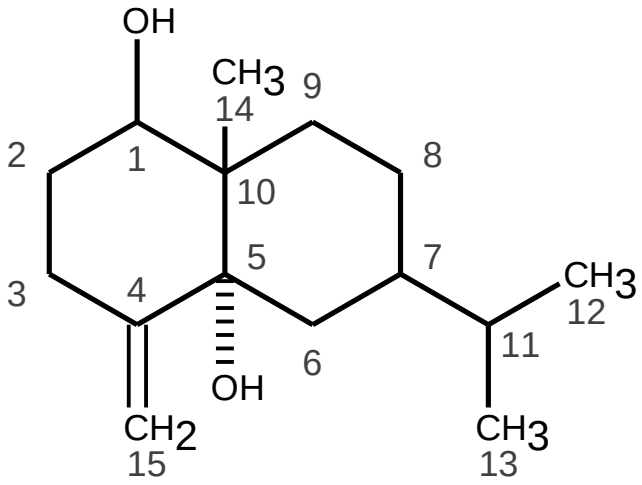
6	4.79(s)	CH	66.7
7	0.94m	CH	48.3
8	1.64m	CH_2	18.7
9	1.20m, 1.89(12.6)	CH_2	38.3
10	-	C	39.4
11	1.65m	CH	28.7
12	1.04d (6.4)	CH_3	21.3
13	0.95d (6.2)	CH_3	22.4
14	1.20 s	CH_3	18.4
15	1.85s	CH_3	17.3

Table 2: ^{1}H NMR and ^{13}C NMR Spectrum data of Compound 2 in $CDCl_3$($400._{MHZ}$ -^{1}H and $100_{MHZ}$for ^{13}CNMR). Multiplicity and Coupling constant (J, Hz) δ ppm.

Position	^{1}H	CH	^{13}C
1	4.07(dd, 12.1, 5.6)	CH	73.6
2	1.76m, 1.60m	CH_2	31.4
3	2.81m, 2.17dd	CH_2	29.6
4	-	C	149.8
5	-	C	75.7
6	1.61, dd, 1.52m	CH_2	33.9
7	1.52m	CH_2	37.5
8	1.29m, 1.63m	CH_2	24.3
9	1.59m, 1.66dd	CH_2	31.2
10	-	C	41.6
11	1.54m	CH	33.5
12	0.94d (6.2)	CH_3	18.5
13	0.91d (6.7)	CH_3	21.0
14	0.78s	CH_3	13.8
15	α 4.87, s β4.76s	CH_2	106.8



Compound I (rel - 1β, 3α, 6β – trihydroxyeudesm – 4 – ene)



Compound II (4 - (15) – eudesmene 1β, 5α – diol.)

Fig.1. Structural Elucidation of compounds I and II

Compound I: This was obtained as an oily optically active material from the n-hexane extract. The IR spectrum of compound 1 shows a strong absorption band at $V_{3423\text{-}1}$ to indicate the presence of hydroxyl group (OH). The medium peak signal at $V_{1385cm\text{-}1}$ is an indicative for the presence of methyl groups [16]. Molecular formula for compound 1 was ascertained as $C_{15}H_{26}O_3$ as established by FABMS data calculated at M/z 254 [17]. The 12 carbon signals were characterized by DEPT experiment showing the presence of four methylene groups on C – 12, C – 13,C – 14 and C – 15, three methylene signals on C – 2, C – 8 and C -9, five methane carbon

signals on C – 1, C – 3, C – 6, C – 7 and C- 11, while three quaternary carbon signals where observed to be found on C – 4, C – 5, and C – 10 respectively [18-19]. Close observation of the ^{1}H NMR and ^{13}C MNR spectral data of compound 1 (Table 1) exhibited the characteristics of sesquiterpenes class commonly found in the genus. The signals observed in the ^{1}H NMR and ^{13}C MNR of compound 1 (Table 1) were also in great conformity with those eudesmane skeleton [20-21]. The HMBC and COSY spectra could be observed to have similar connectivity typical of eusdesmane skeleton [22]. The HMBC and DEPT spectra exhibited a methyl singlet (C – 14) which is been correlated to C – 10 and also to C- 9 (CH_2), C – 5 (quaternary) and also to an oxymethine carbon signal C – 1 [23]. The HMBC spectrum of compound 1 exhibited the attachment of proton signal H – 1 to be a broad doublet (J=12.4) hence therefore an axial [24]. The proton was found to be coupled to the two proton of a methylene group (C – 2) which was further coupled to another oxymethine proton on (H – 3, δ_H= 4.36, δc =84.5) with a broad singlet and which appears to bean equatorial in orientation [25].

The HMBC spectrum of compound 1 shows that, the carbon signal to which this proton was attached to, was also correlated with the protons of a down field methyl group (C – 15) which was also desheilded (δ_H 1.85) due to its direct attachment to a double bond [19]. This correlation was also confirmed by correlation between those methyl protons to a quaternary olefinic carbon on C – 4 and those of the methyl protons of C – 14, hence placing this carbon on C – 5. The most desheilded oxymethine proton (H – 6,δ_H 4.79), also gave HMBC correlations to C – 6. This resonance was also found to exhibit a sharp singlet (equatorial) which is further coupled to a methine proton (H -7) as observed in the COSY spectrum [26]. Signals on H – 7 exhibited further couplings to a methane proton (H – 11), which formed part of the typical eusdesmene sesquiterpenes isopropyl moiety commonly found on C – 7 position and which was further coupled to H – 11 [27]. The coupling constant of δ_C12.40$_{HZ}$ attributed to H – 1 proton was found to be axial and hence lacks any discernible couplings for H – 3 and H – 6 (both singlet) which implies that these protons are equatorial [18]. The accurate mass determination of the compound 1 indicated a molecular formula of $C_{15}H_{26}O_3$ hence suggesting the hydroxyl groups must be placed at C – 1, C – 3 and C – 6 respectively.

Compound II: The Chloroform soluble fraction was submitted to column chromatography over Silica gel with n-hexane / EtOAC (8:0 – 0:1) as eluent to give fractions B_{1-10}. Fraction B_4 was

further resubmitted to a repeated column chromatography using sephadex LH_{20} with MeOH/$CHCl_3$(3:1) to give compound 2 (28 mg).Compound 2 was found to have a molecular formula of $C_{15}H_{26}O_2$ deduced from FABMS [M^+ 238]. The ^{13}CNMR spectra exhibited 15 carbon atom signals with the DEPT experimental showing three methine signals, six methylene carbon signals and three quaternary carbon signals on C –4, C – 5 and the C – 10 [25]. The ^{1}H and^{13}CNMR spectra (Table 2) also revealed an exocyclic double bond attached to C – 4, an isopropyl and two hydroxyl groups attached to C – 1 and C – 5 as established from HMBC [26]. The presence of three methyl protons a tertiary methyl (δ_H0.78, 3H); two isopropyl methyl (δ_H0.94d, 3H, J=6.7H_Z) were also observed [28].

The HMBC and HMQC spectral analysis of compound 2 were also in conformity with those of the eudesmene sesquiterpenes skeleton [29]. The analysis of the coupling constants and NOESY Data of the spectra also established the relative configuration and conformation of compound 2. The NOESY correlation between H – 1 and H – 9 confirms the orientation of the hydroxyl group at C – 1, C – 5 and the C – 14 methyl group as being β, α orientation [26].

CONCLUSION

In conclusion, on the basis of spectral analysis (FTIR, FABMS, 1D and 2D NMR) and comparison with ^{1}H NMR and ^{13}C NMR for reference data, compound I was determined as rel - 1β, 3α, 6β – trihydroxyeudesm – 4 – ene, while Compound II is elucidated to be 4 - (15) – eudesmene 1β, 5α – diol.

Authors contributions

M. Mohammed, A.Abubakar and A.A. Dan mallam participated in the study, design, and critical revision of manuscript for important intellectual content. M. Mohammed, B. Suleiman, D. Ibrahim and M. Salisu drafted the manuscript and participated in analysis and interpretation of the available data.

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