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Isolation and Characterization of α –Amyrin from Stem Bark of *Ficus exasperata* (Vahl)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors LAN and TATA designed the study and wrote the protocol. Author NOU wrote the first draft of the manuscript and managed the analyses of the study. Author JVA managed the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Aims: Isolate and characterize compounds from the ethyl acetate stem bark extract of *Ficus exasperata.*
Place and Duration of Study: Department of Chemistry and Centre for Agrochemical Technology, University of Agriculture Makurdi, Nigeria; Herbarium of the Department of Biological sciences and Department of Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Services of Institute of Leather Research, Zaria, between May 2013 and December 2015.
Methodology: Microwave-assisted extraction was used to extract the stem bark of *Ficus exasperata* using n-hexane, ethyl acetate and methanol. Vacuum Liquid Chromatography was used for isolation from ethyl acetate extract. Proton and Carbon-13 Nuclear Magnetic Resonance spectroscopy were used for characterization.
Results: Vacuum Liquid Chromatography of ethyl acetate crude extract gave rise to fraction N-8, a white amorphous solid a with melting point range of 225-227℃. Proton and Carbon-13 Nuclear Magnetic Resonance spectra data of N-8 was consistent with those published for α-amyrin acetate.

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Conclusion: To the best of our knowledge this is the first report of isolation of α-amyrin acetate from the plant *Ficus exasperata*. Alpha-amyrin acetate has been implicated in a number of studies as having broad spectrum activity against a lot of ails and may well be the leading principle behind *Ficus exasperata*'s various ethnomedicinal applications.

Keywords: Ficus exasperata; ethyl acetate; extraction; α-amyrin acetate.

1. INTRODUCTION

Ficus exasperata is very common in West Africa [1]. It is a small tree with smooth gray bark commonly known as sand paper tree. In Nigeria, the plant is found in secondary rain forest and sometimes besides streams and rivers [2]. It is known by several vernacular names in Nigeria. It is called Eweipin (Yoruba) and Inwalinwa (Igbo) (Southern Nigeria) [3] as well as Hi-tur and Ijakpi, in the Tiv and Igala languages (Central Nigeria), respectively.

Ficus exasperata is used in African traditional medicine for the treatment of several diseases [2]. A decoction of the leaves is used in the treatment of high blood pressure, rheumatoid arthritis, stomach ache, bleeding and epilepsy [4].

A decoction of roots of *F. exasperata* is used in the treatment of Pneumonia in Tanzania [5], aqueous leaf extracts are sprayed on crops as insecticide; leaves and stems to soothe itchy skin [6] among the Igede people of North-central Nigeria, leaves are consumed as vegetable by the Edo people of Nigeria [7]. A decoction of leaves is given as enema or as therapeutic meal to Ndenye women (Eastern Côte d'Ivoire) to maintain foetal integrity during second trimester of pregnancy [8], leaves are used to scrub and clean kitchen utensils in Babungo, Northwest Region of Cameroon [9]. Variously in Nigeria the plant is utilized for treatment of microbial infections, sexually transmitted diseases and gastroenteritis; for controlling high blood pressure, heamostative ophthalmia, coughs and heamorrhoids; young leaves are a common anti-ulcer remedy; anti-diabetic, lipid lowering and antifungal activities; the sap for treating sore eyes and stomach pains [10]. *F. exasperata* is implicated in ritual traditional practices (ethnozoological practices) of the Yoruba of Nigeria [11]. Its' leaves are used in Nigeria to check rancidity in palm oil [12]; young leaves are used as a remedy for ulcer; as well as anti-diabetic and antifungal activities of the leaves have also been reported [1].

It is against this backdrop of enormous ethnobotanic value and lack of commensurate isolations of active principles thereof that we investigate this plant.

We hereby report the isolation and characterization of a triterpenoid from the ethyl acetate stem bark extract of *Ficus exasperata*.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Samples

The stem bark of *Ficus exasperata* was collected from Odogwu in Ibaji Local Government Area of Kogi State (North Central Nigeria). It was identified and authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria with voucher number 2733. Upon collection, the plant sample was washed and air dried under shade for three weeks after which it was pulverized and stored at room temperature until needed for extraction.

2.2 Microwave Assisted Extraction

Microwave assisted extraction (MAE) as described by Iombor and Anyam [13] was carried out using hexane, ethyl acetate and methanol, in that order. The pulverized plant sample (700 g) was extracted with hexane (1.6 L) for 30 minutes (3 minute intervals) using a domestic microwave oven (70 Watts/Defrost Function). After extraction, extracts were allowed to cool to room temperature, filtered and subjected to evaporation at room temperature. This was repeated using ethyl acetate and methanol.

2.3 Vacuum Liquid Chromatography

Ethyl acetate extract (2 g) was pre-adsorbed on Celite (analytical filter aid) for further purification by Vacuum Liquid Chromatography (VLC). The pre-adsorbed sample was air dried. A sintered glass funnel (porosity 3) was loaded with silica gel under vacuum ensuring that it was compacted and uniformly spread to a height of 5 cm. Petroleum ether (40-60) was run through the

column under vacuum then the pre-adsorbed sample was evenly spread on the silica gel. Suction was applied using a vacuum pump. Petroleum ether and Ethyl acetate were used as mobile phase. Gradient elution was employed with the non-polar Petroleum ether as the starting mobile phase and each subsequent mobile phase richer by 1% in the ethyl acetate component until 100% ethyl acetate. Fifty 40 mL fractions (numbered N-1 to N-50) were collected.

Concentrated fractions were subjected to Thin Layer Chromatography (TLC) and similar fractions were pooled together and subjected to repeated VLC with a shorter column. A total of twelve fractions numbered N-1 to N-12 were collected on the basis of TLC similarity. Fraction N-8 gave a white amorphous solid. This was stored at room temperature for further analysis.

Meting point of N-8 was determined by using capillary tube in an oil bath.

Fraction N-8 was characterized using proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and ^{13}C nuclear magnetic resonance (^{13}C-NMR) spectroscopy.

3. RESULTS AND DISCUSSION

Extraction of stem bark of *Ficus exasperata* with ethyl acetate gave 8.9 g of crude extract; percentage yield 1.27%.

VLC of ethyl acetate crude extract gave rise to fraction N-8 as white solid, melting point range of 225-227℃.

^{1}H-NMR and ^{13}C-NMR spectra data of N-8 was consistent with those published for α-amyrin acetate [14–17] (Tables 1 and 2).

N-8 gave ^{1}H-NMR and ^{13}C-NMR signals characteristic of a triterpenoid. The ^{1}H-NMR spectrum of N-8 revealed the presence of several signals between 0.78 and 1.05 (Table 1) which are attributed to overlapping methyl, methylene and methine protons typical of triterpenes. The signal observed at δ 5.11, which is a triplet is typical of an olefinic proton (H-12); that at δ 4.48 corresponds to the oxymethine proton typical of hydrogen at C-3 of triterpenes. This relatively deshielded signal is indicative of substitution of hydroxyl group with acetate group at C-3. An intense signal (a singlet) at δ 2.02 is attributed to methyl protons of the acetate group. The deshielding of this methyl proton was as a result of its proximity to a carbonyl functional group. Crucially, these signals are similar to those reported by other researchers for alpha amyrin acetate. According to Feleke and Brehane [16], the ^{1}H-NMR spectrum of α-amyrin acetate indicated the presence of of two methine protons, one at δ 5.12 (1H, dt) attached to a double bond, and the other at δ 4.5 (1H, m) at position 3; and an acetate group at 2.05 (3H, s) (Table 1).

Table 1. ^{1}H-NMR data of N-8 compared with literature values (400 MHz, $CDCl_3$)

C	N-8(ppm)	[14]	[15]	[16]
1.	-			
2.	-			
3.	4.48 (d)	4.5 (dd)	4.55 (t)	4.5
4.				
5.	-			
6.	-			
7.	-			
8.	-			
9.	-			
10.	-			
11.	-			
12.	5.11(t)	5.12 (t)	5.15 (s)	5.15 (dt)
13.	-			
14.	-			
15.	-			
16.	-			
17.	-			
18.	-			
19.	-			
20.	-			
21.	-			
22.	-			
23.	0.86 (s)	0.88 (s)	-	-
24.	0.86(s)	0.88 (s)	-	-
25.	0.99 (s)	1.01 (s)	0.93 (s)	-
26.	0.96 (s)	0.98 (s)	0.95 (s)	
27.	1.05 (s)	1.07 (s)	1.08 (s)	-
28.	0.78(s)	0.79 (s)	0.80 (s)	
29.	0.83 (s)	0.88 (s)	-	-
30.	0.83 (s)	0.88 (s)	-	-
1^{1}	-	-	-	-
2^{1}	2.02 (s)	2.05 (s)	2.06 (s)	2.05 (s)

Key: s=singlet; dt=doublet of triplets; t= triplet

^{13}C-NMR spectrum of N-8 gave signals that are very similar to those reported for alpha-amyrin acetate. ^{13}C-NMR signal at δ 171.07 is attributed to carbonyl carbon of an acetate group attached to C-3 of the alpha amyrin, while the signal at δ 81.03 indicated oxymethine carbon (C-3) which was slightly deshielded due to its attachment to the acetate group. The signals at δ 124.39, assigned to C- 12, and δ 139.70, assigned to C-13 are indicative of olefinic carbons at C-12 and

C-13. Also, there was an observed carbon signal δ 59.13 which corresponds to the methine carbon, C-18 of alpha-amyrin moiety. All these assignments were consistent with the data obtained from literature for alpha-amyrin acetate [14,16,17] (Table 2).

Table 2. ^{13}C-NMR of Data of N-8 Compared with Literature Values (100 MHz, $CDCl_3$)

C	N-8(ppm)	[17]	[16]	[14]
1.	38.46	38.4	38.8	38.5
2.	23.45	23.86	28.5	23.4
3.	81.03	80.8	81.3	80.9
4.	37.78	37.94	38.1	37.7
5.	55.33	55.40	55.6	55.3
6.	18.32	18.39	18.6	18.3
7.	32.94	32.89	33.2	32.9
8.	39.72	39.6	37.2	39.7
9.	48.08	47.67	48.0	47.7
10.	36.86	36.6	34.1	36.8
11.	23.45	21.07	23.6	22.8
12.	124.39	124.2	124.7	124.2
13.	139.70	139.5	140.0	139.5
14.	42.14	42.0	42.4	42.1
15.	28.15	27.58	28.5	28.2
16.	26.68	26.72	27.0	26.7
17.	33.82	33.7	33.2	33.8
18.	59.13	59.0	59.4	59.0
19.	39.72	39.6	40.0	39.7
20.	40.8		40.0	39.7
21.	31.33	31.2	31.6	31.3
22.	41.6	41.5	41.9	41.6
23.	28.15	14.53	28.4	28.1
24.	16.05	28.09	16.1	15.8
25.	16.26	16.33	17.1	14.2
26.	16.83	18.84	17.2	16.8
27.	19.32	19.32	23.6	17.6
28.	28.83	18.05	28.4	28.8
29.	17.59		17.9	23.3
30.	21.49	19.47	21.7	21.4
1^1	171.07	171.02	171.4	170.8
2^1	21.49	21.42	21.7	21.5

Alpha-amyrin acetate has been isolated from other members of the *Ficus* genus: including *F. religiosa*, *F. racemosa* and *F. bengalensis F. chlamydocarpa* [18]; *F. pandurata* [19]; *F. pseudopalma* and *F. ulmifolia* [20] and *F. nota* [21].

α-amyrin acetate is reported to possess anti-erectile dysfunction effects [22] anti-inflammatory [23–25] analgesic properties [26,27], growth inhibitory effect on Streptococcus [28], suppressive effects on male albino rat fertility [29], antihyperglycaemic [30], larvicidal [31], cytotoxic and antispasmodic [14], apoptosis of leukemia cells [32], antihepatoxic and antioxidant [18], a molecular docking study found alpha-amyrin to be weakly docking with human estrogen receptors β (ERβ) and hence unlikely to bind to the human estrogen receptor and exhibit selective estrogen receptor modulation [33]. Recent evidence indicates that α-amyrin probably displays its effects through interaction with the cannabinoid pathway [25]; another study has also indicated evidence for participation in protein kinase C and protein kinase A pathways [26].

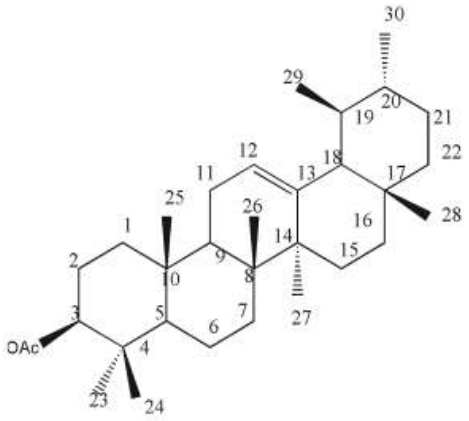

Fig. 1. Structure of α-Amyrin acetate

4. CONCLUSION

Encouraged by the numerous applications of *Ficus exasperata* in African traditional practices, stem bark extracts were investigated; that α-amyrin acetate was isolated supports some ethnomedicinal applications of the plant. The study is also of chemotaxonomic relevance as α-amyrin acetate is a leading principle in the Genus *Ficus*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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