UJPICR Volume 2 Number 1 :June, 2022
UMYU Journal of Pure and Industrial Chemical Research
Volume 2 No. 1 June, 2022.
ISSN: **2814 – 1628/2814 - 1636**
DOI: https://doi.org/10.55688/ujpicr21.008

ISOLATION OF B-AMYRIN ACETATE FROM THE LEAF OF *Ficus sycomorous L.* (Moracaea)

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Abstract

Ficus sycomorous (Family: Moraceae) is a plant used in African traditional medicine to treat mental illness, dysentery, cough, diarrhea, tuberculosis and cancer. The aim of this study is to identify and characterize some compounds from the leaf extract of the plant. The dried pulverized leaf of the plant was extracted using dichloromethane for 72 hours. The extract was drained and filtered. The DCM extract was subjected to Flash Column Chromatography using mobile phase, which progressed from 100% n- hexane to 1:1 mixture of Dichloromethane and ethyl acetate and Silica gel (60-120) as stationary phase. This resulted in the isolation of β-amyrin acetate. The structures of these compounds were established by careful analysis of their spectral (^{1}H, ^{13}C and 2D NMR) data and comparing them with those reported in the literature.

Keyword: *Ficus sycomorous*, Moraceae, β-amyrin acetate, NMR

INTRODUCTION

Medicinal plants, either through systematic screening programs or by serendipity, possess an important position in the drug discovery. Many modern drugs have their origin in traditional medicine from different cultures[1]. Ethnopharmacological data are obtained not only by consulting traditional healers and accumulating information on the popular medicinal use of plants, but also from literature on folk medicine[2]. Furthermore, Elujoba[3] noted that a plant becomes a medicinal plant only when its biological activity has been ethnobotanically reported or scientifically established.

Moraceae, often called the mulberry family, is a family of flowering plants comprising of about 40 genera and over 1000 species of which over 500 species are members of the genus *Ficus*[4]. They occur primarily in

tropical and semi-tropical regions, and include a wide variety of herbs, shrubs, and trees, characterized by a milky sap[5,6].

Ficus is the Latin name for fig, derived from the Persian 'fica'. In Greek 'syka' means fig. The name of the species comes from the Greek 'sykamorea' (sycamore), used in the Gospel according to St. Luke; it was the tree that Jesus cursed because it was barren[6]. *Ficus sycomorus* is a large, semi-deciduous spreading savannah tree, up to 21 (max. 46) m in height; it is occasionally buttressed. Its leaves are broadly (ob) ovate or elliptic, the sub base is cordate, the apex is rounded or obtuse, the margin is entirely or slightly repand-dentate (2.5-13 (max. 21) x 2-10 (max. 16 cm) and is scabrous above; the petiole is 1-5 cm long, with five to seven pairs of yellow lateral veins; the lowest pair originates at the leaf base. It can be monocious or diecious[7]. Bark on young stems is pale green with a soft powdery covering; on older stems, grey-green, fairly smooth, with scattered grey scales and pale brown patches where scales have fallen off. When on the tree it produces heavy latex flow[8] The plant is widely distributed in tropical Africa stretching from Senegal to South Africa including Nigeria, Niger, Mali, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast. In Nigeria the plant is mostly found in semi-arid regions. This savannah tree usually grows in high water table areas. It is often found along watercourses such as streams and rivers, swamps and waterholes[6]. The plant is commonly known as Sholla or Bamba in Amharic (Ethiopia); large-fruited sycamore fig (English), Mukuyu (Shona), Mukuyukono (Shona), Muonde (Shona), Musvunguzu (Shona), Mutsvita (Shona), Umkhiwa (Ndebele), Baure (Hausa, Northern Nigeria), Tarmu (Kanuri), and Kamda (Babur/Bura), among others[9]. In northern Nigeria, the stem bark of *Ficus sycomorus* is used traditionally to treat fungal diseases, jaundice and dysentery[7]. The Hausa and Fulani tribes of northern Nigeria use the stem-bark of *F. sycomorus* to treat diabetes mellitus, fungal diseases, jaundice and dysentery[10,11]. The parts of *Ficus sycomorus* used traditionally for the treatment of tumors and diseases associated or characterized by inflammation include the fruits in different stages of ripening, fresh or dry, tree bark, leaves, twigs and young shoots, and also latex from the bark, fruit and young branches[12].

Despite the vast use of different parts of the plant *F. syncomorus* in traditional medicine to treat so many diseases, there is paucity if literature on the Chemistry of the plant. The

main aim of this research work therefore, is to isolate and characterize some compound(s)from leaves of *F. syncomorus*

Materials and Methods

Collection and identification of Plant Material

The leaves of *Ficus sycomorus* were collected in the month of April, 2013, from Turunku village, Igabi local government area, Kaduna state. The plant was identified by Mallam U.S. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria, (voucher specimen number 1466). The plant material was air dried under shade until constant weight was obtained and size reduced manually using clean mortar and pestle.

Extraction

The powdered plant material (2.5 Kg) was subjected to cold maceration using dichloromethane (DCM) and methanol with occasional shaking for three days. The extract was drained and filtered using Whatman (No1) filter paper, concentrated using rotary evaporator and allowed to dry *in-vacuo*. This yielded a dark green residue (73.5g) referred to as Dichloromethane Extract (DE). The marc from the DCM extract was further extracted by the same method using methanol (100%). This yielded a dark brown residue (70.2g) referred to as Methanol Extract (ME). The DCM extract was used for this research.

Chromatographic separation

The DCM extract was subjected to Flash Column Chromatography (referred to as the FCC). 20g of silica gel (mesh size: 60-120) was added to the leaf extract in dichloromethane (DCM). The mixture was dried and grinded into a fine powder. The powder was loaded into a sample cartridge and fitted to the FCC instrument, along with a 150g sample-mass- cartridge packed with silica gel (mesh size: 60-120). Gradient elution was used such that the mobile phase progressed from 100% n-hexane to a 1:1 mixture of DCM and ethylacetate. A total of 150 fractions (45mls each) were collected into individual 100ml beakers. Fractions which displayed similar retention factor (R_f) values with the same solvent mixture from TLC analysis and similar ^{1}H NMR spectrum were combined for further purification. A combination of column chromatography over both silica gel (60-120 mesh) and Sephadex **(**LH-20) were used for purification of the pooled fractions.

The DCM FCC fraction 48 which showed one prominent spot and traces of impurities was subjected to silica gel column chromatography eluted with hexane :

ethylacetate (9:1). Thirty five collections were made. Collections 3 and 4 showed a single homogenous spot on TLC chromatogram using the same solvent system. The purity and similarities of these collections were further confirmed using their proton NMR spectrum. They were combined and coded A5.

Plate 1: TLC chromatogram of A5

RESULTS

The LCMS of A5 displayed a $[M-H]^+$ ion peak at *m/z* 467.

The IR spectrum of A5 showed absorptions bands at 2921 and 2851 cm^{-1} (C-H stretching), 1737 cm^{-1} (C=O stretching) 1636 cm^{-1} (C=C stretch) and 1244 cm^{-1} (C-O stretching).

The ^1H NMR (500 MHz, $CDCl_3$) spectrum of A5 showed proton signals at δ 0.84 (s, 3H-29), 0.89 (d, *J* = 7 Hz, H-30), 1.08 (d, *J* = 7.0 Hz, H-27), 1.27–1.30 (d, *J* = 7.0 Hz,), 1.48 (m, H-21), 1.52 (d, *J* = 11.5 Hz, H-18), 1.69 (s, 3H-2, 3H-7 and 3H-5), 1.83 (m, 2H-19), 1.86 (m, 2H-22), 1.90 (m, 2H-15), 2.05 (d, *J* = 11.5 Hz, H-1), 2.19 (s, H-9), 2.23–2.26 (d, *J* = 11.5 Hz, H-1 and H-16), 5.14 (s, 2H-11) and 5.55 (s, H-12).

The ^{13}C NMR spectrum of A showed a total of thirty-two carbon resonances at δc 39.6 (C-1), 27.9 (C-2), 80.8 (C-3), 39.6 (C-4), 55.2 (C-5), 18.2 (C-6), 33.7 (C-7), 38.4 (C-8), 47.4 (C-9), 35.6 (C-10), 23.6 (C-11), 121.6 (C-12), 145.2 (C-13), 42.1 (C-14), 28.7 (C-15), 27.9 (C-16), 32.9 (C-17), 59.0 (C-18), 40.0 (C-19), 41.5 (C-20), 31.2 (C-21), 42.0 (C-22), 29.7 (C-23), 15.7 (C-24), 15.7 (C-25), 16.8 (C-26), 23.6 (C-27), 28.7 (C-28), 17.5 (C-29), 21.4 (C-30), 170.8 and 21.3 (OAc). While the 2D-NMR data was presented in Table 1

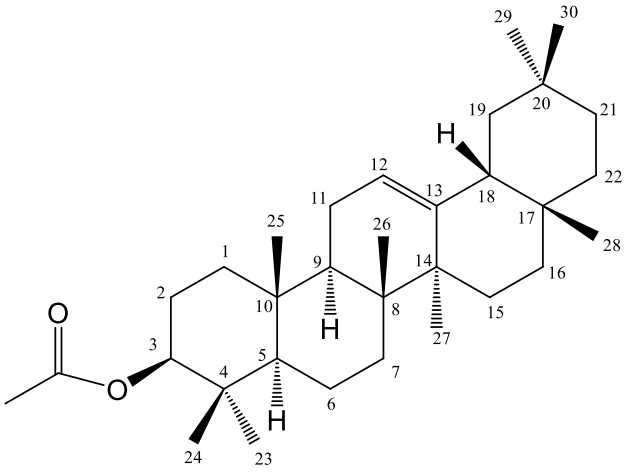
Table 1: NMR spectral data of compound A5 compared compared with literature

No.	^{13}C NMR (500 MHz)	^{13}C NMR (500 MHz) in $CDCl_3$ Lee *et al.*, (2014)	^1H NMR (500 MHz)	1H NMR (500 MHz) in $CDCl_3$ Lee *et al.*, (2014)	HMBC (H → C)	COSY (H → H)	NOESY (H → H)
1α	38.5 CH_2	38.3	1.06 m	1.04	-	1β, 2	1β, 2, 3, 9

1β			1.63 m	1.64	3, 10	1α, 2	1α, 3, 11, 25
2	23.76 CH_2	23.5	1.63 m	1.88	3, 4, 10	1α, 1β, 3	1α, 3, 25
3	81.2 CH	81.0	4.5 dd*J*=10.0, 5.5 Hz	4.50 *t J*=8.0 Hz	-	2	1α, 1β, 2, 5, 9
4	37.9 C	39.8	-	-	-	-	-
5	55.5 CH	55.2	0.84 m	0.84 *m*	7, 10, 23, 24, 25	6α, 6β	3, 9, 6α, 6β, 7α
6α	18.5 CH_2	18.3	1.55 m	1.53 *m*	-	5, 6β, 7β	5, 6β, 7β, 27
6β			1.40 m	1.40 *m*	-	5, 6α, 7α, 7β	5, 6α
7α	32.8 CH_2	32.6	1.50 m	1.52 *m*	-	6β, 7β	5, 7β, 27
7β			1.34 m	1.33 *m*	-	6α, 6β, 7α	6α, 7α, 26
8	40.0 C	37.7	-	-	-	-	-
9	47.8 CH	47.6	1.58 m	1.58 *m*	-	11	1α, 3, 5, 27
10	37.1 C	36.8	-	-	-	-	-
11	23.8 CH_2	23.6	1.87 m	1.80 *m*	-	11, 12	1β, 12, 25
12	121.9 CH	121.6	5.18 t *J*=3.7 Hz	5.18 t J=3.5 Hz	9, 18	11	18, 26
13	145.5 C	145.2	-	-	-	-	-
14	41.9 C	41.7	-	-	-	-	-
15α	26.4 CH_2	26.1	1.78 m	1.76 *m*	-	15β, 16α, 16β	15β, 25, 28
15β			0.96 m	0.85 *m*	8, 14, 17	15α, 16α	15α, 16β, 18
16α	27.2 CH_2	26.9	2.0 m	1.98 *m*	-	15α, 15β, 16β	16β, 27, 28
16β			0.8 m	0.79 *m*	14, 17, 28	15α, 16β	15β, 16α, 18, 26
17	32.7 C	32.5	-	-	-	-	-
18	47.5 CH	47.2	1.96 m	1.93 d *J*=4.2 Hz	-	19α, 19β	12, 15β, 16β, 19β, 26, 28, 30
19α	47.0 CH_2	46.8	1.01 m	1.00 *m*	17, 18, 29	18, 19β	19β, 21α
19β			1.66 m	1.66 *m*	20, 29, 30	18, 19α	18, 19α, 21β, 26
20	31.3 C	31.1	-	-	-	-	-
21α	35.0 CH_2	34.7	1.34 m	1.32 *m*	-	21β, 22β	19α, 21β, 27
21β			1.10 m	1.08 *m*	-	21α, 22β	19β, 21α, 22β, 28
22α	37.4 CH_2	37.1	1.21 m	-	-	22β	22β
22β			1.41 m	-	-	21α, 21β, 22α	21β, 22α, 28
23	28.3 CH_3	28.0	0.87 s	0.88 s	3, 4, 5, 24	-	-
24	16.9 CH_3	15.6	0.86 s	0.86 s	3, 4, 5, 23	-	-
25	15.8 CH_3	15.7	0.98 s	0.96 s	1, 5, 9, 10	-	1β, 2, 15α, 28

26	17.0 CH_3	16.8	1.00 s	0.97 s	7, 8, 9, 14	-	7β, 16β, 18, 19β, 28
27	26.2 CH_3	26.0	1.13 s	1.13 s	8, 13, 14, 15	-	6α, 7α, 9, 16α
28	28.6 CH_3	28.4	0.83 s	0.83 s	16, 17, 18, 22	-	15α, 18, 21β, 22β, 25, 26
29	33.6 CH_3	33.4	0.87 s	0.87 s	19, 20, 21, 30	-	-
30	23.9 CH_3	23.7	0.87 s	0.87 s	19, 20, 21, 29	-	18
O-CO-CH_3	171.4 C	171.1	-	-	-	-	-
O-CO-CH_3	21.5 CH_3	21.4	2.05 s	2.05 s	O-CO-CH_3	-	

DISCUSSION



Compound **A5,** isolated as white oil was found to be β-amyrin acetate.

The ESI-MS spectrum of A5 showed a $[M-H]^+$ ion peak at *m/z* 467.3903which indicated a molecular formula of$C_{32}H_{52}O_2$for the compound. A double bond equivalence of seven was calculated for this compound. The IR spectrum showed absorptions bands at 2921 and 2851 cm^{-1} for C-H stretches, 1737 $cm^{-1}$for the carbonyl stretch of an acetate group, 1636 cm^{-1} for a double bond stretch and 1244 cm^{-1} for a C-O stretch.

The ^1H NMR spectrum showed a resonance at δ_H 4.50 (dd, J = 10.0, 5.9 Hz), which corresponded to the carbon resonance at δ_c 81.2 in the HSQC spectrum. The observed coupling constants suggested that the configuration of H-3 was α with the acetate group in the β-position.

The ^{13}CNMR spectra of A5 displayed thirty-two carbon resonances which according to the DEPT-135 spectrum included nine methyl, ten methylene, six methine, and seven fully substituted carbons resonances. This indicated that compound A5 was an acetylated pentacyclic triterpenoid derivative**.** The HMBC spectrum of A5 showed correlation between the methine proton resonance at δ_H 0.84 with the two methyl group carbon resonances at δc 28.3 (C-23) and δ_H 16.9 (C-24). Similarly, the olefinic proton resonance, H-12 (δ_H 5.18, t, *J*=3.7) showed correlations with the C-9 (δ_c 47.8) and C-18 (δ_c 47.5) carbon resonances, and it showed further coupling with the H-11 (δ_H 1.87) resonance in the COSY spectrum. The HMBC correlation between the proton resonance at δ_H 1.13 (3H-27) and δ_c 145.5 (C-13) confirmed this placement for the alkene group.

In the NOESY spectrum , the H-3 resonance at δ_H 4.50 showed correlations with the H-5 (δ_H0.84) and H-9 (δ_H1.58) resonances. The H-9 resonance showed also correlations with the H-5 (δ_H 0.84) and 3H-27 (δ_H 1.13) resonances. All these protons (H-5, H-9, 3H-27) are in the α-orientation according to the biosynthesis, while the H-18 (δ_H 1.96), 3H-25 (δ_H0.98) and 3H-26 (δ_H 1.00) resonances, which showed correlations with each other in the NOESY spectrum, are in the β- orientation[13]. The NMR data compared well with literature values[14]. Previous research showed that β-amyrin acetate inflammatory activity[15], sedative, anxiolytic and anticonvulsant properties[16]. Previous research showed thatβ-amyrin acetate inflammatory activity[15], sedative, anxiolytic and anticonvulsant properties[16].

Conclusion

In this study β-amyrin acetate, a compound with wide range of biological activities, had been successfully isolated from the leaves of *Ficus sycomorus* sing chromatographic technics and its structure established using spectroscopic technics such IR and NMR. To the best of our search the isolation of this compound from the plant is reported here for the first time.

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