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Isolation of squalene and its derivative from the leaf of *Ficus sycomorous* L. (Moracaea)

Atiku I.*, Pateh U.U., Sule M.I. and Isyaka M.S.

**Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University Zaria, Nigeria*

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 squalene and squalenol. 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.

Keywords: *Ficus sycomorous*, Moraceae, Squalene, Squalenol, NMR

Full length article **Corresponding Author*, e-mail: chematiku@gmail.com and iatiku@abu.edu.ng

1. Introduction

Squalene is a very valuable compound commonly found in vegetables and animal cells, because it is an intermediate in phytoesterols and cholesterol biosynthetic-pathways and highly appreciated by its biological importance [1]. Squalene is a hydrocarbonated chain ($C_{30}H_{50}$), a triterpene containing six unsaturated bonds with antioxidant nature [2]. Squalene has applications in various end-user industries such as cosmetic, food supplements, pharmaceuticals, and in other applications like high grade lubrication and fiber coating additives. However, the major data of commercial is referred to Shark Liver Oil (SLO). In USA SLO was used for vitamin A production but now is highly recommended in alternative medicine and ointment [3].

Ficus sycomorus is a medicinal plant which belongs to the family Moraceae [4]. It is a large, semi-deciduous spreading savannah tree, up to 21 (max. 46) m. Its leaves are broadly ovate or elliptic, the sub base is cordate, apex is rounded or obtuse and is scabrous above; petiole is 1-5 cm long, with five to seven pairs of yellow lateral veins; lowest pair originates at the leaf base. The plant is widely distributed in tropical Africa stretching from Senegal to South Africa. The plant is found growing in Nigeria, Niger, Mali, South Africa, Guinea, Kenya, Tanzania, Somalia, Ethiopia and Ivory Coast. In Nigeria, the plant is mostly found in semi-arid regions [5]. The plant is referred to by number of local names: It is commonly known as Sycamore fig. (English), Baure (Hausa), Tarmu (Kanuri), and Kamda (Babur/Bura) among others [6].

Different parts of the plant are used traditionally to treat various ailments such as tumors and diseases associated or characterized by inflammation. These include the fruits in different stages of ripening, fresh or dry, stem bark, leaves, twigs and young shoots, and also latex from the bark, fruit and young branches [7]. El-Sayed *et al.*, 2010 [8] reported the isolation of quercetin gallic acid quercetin 3-*O*-L-rhamnopyranosyl(1→6)-β-D-glucopyranoside (Rutin), quercetin 3-*O*-β-D glucopyranoside (Isoquercitrin), quercetin 3,7-*O*-α-L-dirhamnoside, quercetin 3-*O*-β-D-galactopyranosyl(1→6)-glucopyranoside and β-sitosterol-3-β-glucopyranoside from the leaf of this plant. To the best of our search no report was found on the isolation of squalene or squalenol from the leaf of *Ficus sycomorus*.

2. Materials and methods

2.1. 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.

2.2. Extraction

The powdered plant material (2.5 Kg) was subjected to cold maceration using two different solvents: dichloromethane (DCM) and methanol with occasional shaking for three days. The extract was drained and filtered using Whatman (No.1) filter paper, concentrated using rotary evaporator and was 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.

2.3. 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 ethyl acetate. 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 Flash column bulk fraction 18-21 which showed two spots on TLC chromatogram was subjected to silica gel column chromatography and was eluted isocratically with n-hexane:ethylacetate (9:1). Thirty-one collections were made. Collections 12-14 both showed single homogenous spot on TLC chromatogram using the same solvent system. Their purities were also confirmed using their proton NMR spectrum. They were combined and coded A1. The DCM FC bulk fraction 33-38 was subjected to repeated gel filtration using sephadex LH20 eluted with DCM:methanol (1:1). Collections 6-8 of the third running which gave single homogenous spot on TLC chromatogram using the same solvent system and had the same proton NMR spectra were combined together and coded A2.

3. Results

Compound A1 (15 mg) was isolated as a yellowish oil with an R_f value of 0.876 using n-hexane:ethylacetate (9:1) as solvent system (Plate 1).

Plate 1: TLC chromatogram of A1 using n-hexane:ethyl-acetate 9:1 as solvent system

The ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of A1 showed methyl groups resonances at δ_H 1.60 (s, 18H) and δ_H 1.68 (s, 6H), methylene groups resonances at δ_H 1.99-2.03 (m, 20H), and internal olefinic signals at δ_H 5.06-5.15 (m, 6H).

The ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of A showed signals at δc 25.70 (C-1/4), 131.2 (C-2/23), 124.2 (C-3/22), 39.8 (C-5/20), 134.8 (C-6/9), 124.4 (C-7/18), 26.6 (C-8/17), 135.0 (C-10/15), 124.3 (C-11/14), 28.3 (C-12/13), 17.6 (C-25/30), 16.0 (C-26/29), and 16.0 (C-27/28). The DEPT-135 spectrum of A1 revealed eight methyl, ten methylene and six methine carbon resonances. Six quaternary carbons which disappeared in the spectrum were also noted.

3.1. Isolation of A2

Compound A2 (4.6 mg) was isolated as yellowish oil. It had an Rf = 0.47 using n-hexane:ethylacetate (8:2) as solvent system (Plate 2).

Plate 2: TLC Chromatogram of A2 using n-hexane : ethyl-acetate (8:2) as solvent system

The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of A2 showed methyl group proton resonances at δ_H 1.25 (s, 6H), δ_H 1.61 (s, 6H), and δ_H 1.740 (d, J = 1.25 Hz, 6H) overlapping methylene groups resonances at δ_H 1.97- 2.09 (m, 20H), and olefinic proton signals at δ_H 5.11-5.13 (m, 5H), carbinol and another upfield olefinic proton signal at δ_H 5.44 (ddd, J = 1.4 Hz, 7.2 Hz, H-7) and δ_H 4.09 (d, J = 5.3 Hz, 2H-26).

The ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of A2 showed carbon resonances δc 25.9 (C-1/4), 131.4 (C-2/23), 124.3 (C-3/22), 26.7 (C-4), 39.9 (C-5/20), 135.2 (C-6), 124.5 (C-7), 26.9 (C-8/17), 39.91 (C-9), 135.1 (C-10/15), 124.4 (C-11/14), 27.3 (C-12/13), 17.6 (C-25/30), 59.2 (C-26), 16.1 (C-27/28) and 26.0 (C-29).

From the DEPT-135 spectrum of A2 revealed seven methyl, eleven methylene and six methine carbon resonances. Six quaternary carbons which disappeared in the spectrum and other additional peaks were also noted.

The COSY spectrum of A2 showed important long range correlations between proton resonance at δ_H 4.01 and the methine proton at δ_H 5.45. It also showed correlations between proton resonance at δ_H (H-4) and δ_H (H-5); δ_H (H-20) and δ_H (H-21) and between δ_H (H-13) and δ_H (H-14).

Table 1: NMR data of A1 compared with literature
*Refers to overlapping resonance

No	^{13}C NMR (400 MHz) $CDCl_3$	^{13}C NMR (500 MHz) $CDCl_3$ [9]	^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)	^{1}H NMR Lit (400 MHz) $CDCl_3$ [9]
1/24	25.7 CH_3	25.7	1.68 s*	-
2/23	131.2 C	131.2	-	
3/22	124.2 CH	124.4	5.11 m*	5.07-5.13*
4/21	26.8 CH_2	26.7	2.07 m*	2.08
5/20	39.8 CH_2	39.7	1.98 m*	1.94
6/19	134.8 C	134.9	-	
7/18	124.4 CH	124.3	5.11 m*	5.07-5.13
8/17	26.6 CH_2	26.6	2.07 m*	2.08
9/16	39.7 CH_2	40.0	1.98 m*	1.94
10/15	135.0 C	135.1	-	
11/14	124.3 CH	124.2	5.11 m*	5.07-1.13
12/13	28.3 CH_2	28.2	2.01 dd *J* (3.3, 6.8)	2.08
25/30	17.6 CH_3	17.6	1.60 s*	1.58
26/29	16.0 CH_3	16.0	1.60 s*	1.58
27/28	16.0 CH_3	16.0	1.60 s*	1.58

Table 2: NMR spectral data of A2

No	^{13}C NMR (400 MHz) $CDCl_3$	^{1}H NMR (500 MHz) $CDCl_3$ (*J* in Hz)	HMBC C→H	COSY H→H
1/24	25.9 CH_3	1.68 s*		
2/23	131.4 C	-		
3/22	124.3 CH	5.12 m*		
4	26.7 CH_2	2.07 m		5
5/20	39.9 CH_2	2.00 m*		4

6/19	134.8	C	-		
7	135.2	CH	5.44 m	5,6,26	26
8/17	26.9	CH_2	2.07 m*		29
9	39.9	CH_2	2.00 m*		
10/15	135.1	C	-		
11/14	124.4	CH	5.12 m*		
12/13	27.3	CH_2	2.01 dd, (3.3, 6.8)		
25/30	17.6	CH_3	1.70 s		
26	59.2	CH_3	4.09 d, (5.3)	5,6,7	7
27/28	16.0	CH_3	1.68 s*		
29	26.0	CH_3	1.68*	5,7	18

*Refers to overlapped proton resonances

4. Discussion

4.1. Spectral analysis of A1

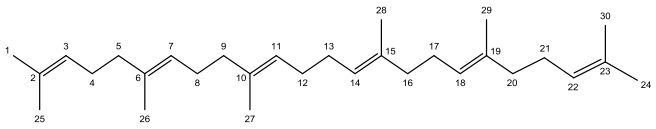

Fig.1. Compound A1 identified as squalene, an acyclic triterpenoid

The GC-MS spectrum of compound A showed a molecular ion peak M^+ at *m/z* 410 which corresponded to molecular formula $C_{30}H_{50.}$ The ^1H NMR spectrum showed six olefinic proton resonances between δ_H 5.08-5.15 which were assigned to H-3, 7, 11, 14, 18, and 22; ten methylene proton resonances between δ_H 1.96 and δ_H 2.10 and eight methyl groups between δ_H 1.25 and 1.68.

The ^{13}C NMR spectrum displayed fifteen carbon resonances representing thirty carbons indicating that the molecule was symmetrical. These include eight methyl carbons (between δc 16.2 and 17.9), ten methylene carbons (between δc 25.9 and 40.0), and six vinyl methine carbons (between δc 124.5 and 135.3).

The ^1H and ^{13}C NMR spectral features of this compound were comparable with those reported for squalene by Consolacion *et al*., 2015 [9] (Table 1). This was further confirmed by a search for molecular ion peak of the compound and its fragmentation pattern in the NIST standard reference library of the GC-MS instrument and was found to (99%) match that of squalene. Hence compound A1 was confirmed to be squalene.

Based on our literature search this is the first report of squalene from this plant. However, the compound was previously isolated from *F. pseudopalma, F. linearifolia, F. triangularis, F. odorata*, and *F. nervosa* [10]. This compound was also reported to be isolated from stem bark and leaves of *Ficus sarmentos* [11], and *F. Minahassae* [12]. Squalene, a precursor to the synthesis of terpenoids and phytosterols, has been reported to possess cardioprotective effects which are related to inhibition of lipid accumulation and/or its antioxidant properties [13]. It was reported that the compound significantly suppresses azoxymethane-induced colonic aberrant crypt foci (ACF) formation and crypt multiplicity which could lead to possible chemopreventive activity against colon carcinogenesis [14].

Squalene is a structurally unique triterpene compound that is one of the main components (about 13%) of skin surface lipids [15]. It is transported in serum generally in association with very low density lipoproteins and is distributed ubiquitously in human tissues, with the greatest concentration in the skin. Squalene is not very susceptible to peroxidation and appears to function in the skin as a quencher of singlet oxygen radicals, protecting human skin surfaces from lipid peroxidation due to exposure to UV light and other sources of oxidative damage [16]. Experimental studies have shown that squalene can effectively inhibit chemically induced skin, colon, and lung tumorigenesis in rodents [17].

4.2. Spectral analysis of A2

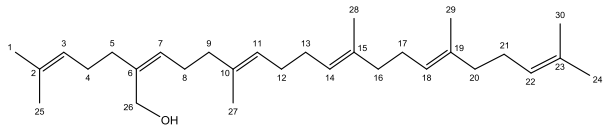

Fig.2. Compound A2 was identified as squalenol

Most of the NMR spectral data of A2 matched with those of compound A1 (squalene) except that A2 is not symmetrical and was found to be more polar than A1, hence more than fifteen carbon resonances were observed in the ^{13}C NMR spectrum. Similarly, an additional carbon resonance was observed at δ_c 59.0 shown by the HSQC-DEPT spectrum to be correlated to the proton resonance at δ_H 4.09. The down field shift observed for C-6 (δ_c 140.1) and H-7 (δ_H 5.45) as well as the HMBC correlation between

that carbon resonance and the carbinol protons suggested that the additional $–CH_2OH$ groups may be attached at position C-6. However considering the similarities of chemical environment in the structure of squalene, this group could be attached at other similar positions such as C-10, C-15 and C-19. This explained the additional (overlapping) carbon resonances observed in the ^{13}C NMR spectrum δ_c 124.4 - 125.2, 135.1 - 136.2 and 25.9 - 26. The C-H and H-H connectivities were established from the HMBC spectrum and COSY spectrum respectively. A long COSY correlation was observed between the carbinol proton δ_H 4.01 (2H-26) and the methine proton at δ_H 5.45 (H-7). Using these spectral data, compound A2 was identified as squalenol. To the best of our literature search this is the first report of this compound.

References

[1] Z.-R. Huang, Y.-K. Lin, J.-Y. Fang. (2009). Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules. 14(1): 540-554.

[2] D.A. Rose. (1996). An overview of world trade in sharks and other cartilaginous fishes. A Traffic Network report.

[3] L.H. Reddy, P. Couvreur. (2009). Squalene: A natural triterpene for use in disease management and therapy. Advanced drug delivery reviews. 61(15): 1412-1426.

[4] S. Van Noort, A. Gardiner, K. Tolley. (2007). New records of Ficus (Moraceae) species emphasize the conservation significance of inselbergs in Mozambique. South African Journal of Botany. 73(4): 642-649.

[5] C. Williams, W. Chew, J. Rajaratnam. (1980). Tree and field crops of the wetter regions of the tropics. Longman Group Ltd.: pp.

[6] M. Hyde, B. Wursten, P. Ballings, Flora of Zimbabwe: Species information: *Kirkia acuminata*. In 2013.

[7] E.P. Lansky, H.M. Paavilainen, A.D. Pawlus, R.A. Newman. (2008). Ficus spp.(fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. Journal of Ethnopharmacology. 119(2): 195-213.

[8] M.M. El-Sayed, M.A.-A. Mahmoud, H.A.-K. El-Nahas, S.A.-H. El-Toumy, E.A. El-Wakil, M.A. Ghareeb. (2010). Bio-guided isolation and structure elucidation of antioxidant compounds from the leaves of *Ficus sycomorus*. Pharmacologyonline. 3(3): 317-32.

[9] C.Y. Ragasa, M.C.S. Tan, D.R. Fortin, C.-C. Shen. (2015). Chemical constituents of *Ixora philippinensis* Merr. Journal of Applied Pharmaceutical Science. 5(09): 062-067.

[10] W.A. Mustaqim, W.H. Ardi. (2020). *Ficus minahassae* (Teijsm. & de Vriese) Miq. Moraceae. Ethnobotany of the Mountain Regions of Southeast Asia. 1-6.

[11] X.-g. WANG, X.-y. WEI, X.-y. HUANG, L.-t. SHEN, Y.-q. TIAN, H.-h. XU. (2011). Insecticidal constructure and bioactivities of compounds from *Ficus sarmentosa* var. henryi. Agricultural Sciences in China. 10(9): 1402-1409.

[12] C.Y. Ragasa, M. Macuha, M.M. De Los Reyes, E.H. Mandia, I.A. Van Altena. (2016). Chemical constituents of *Ficus* septica Burm. F. International Journal of Pharmaceutical and Clinical Research. 8(11): 1464-1469.

[13] K.S. Farvin, R. Anandan, S.H.S. Kumar, K. Shiny, S. Mathew, T. Sankar, P.V. Nair. (2006). Cardioprotective effect of squalene on lipid profile in isoprenaline-induced myocardial infarction in rats. Journal of Medicinal Food. 9(4): 531-536.

[14] C.V. Rao, H.L. Newmark, B.S. Reddy. (1998). Chemopreventive effect of squalene on colon cancer. Carcinogenesis. 19(2): 287-290.

[15] S. Passi, O. De Pità, P. Puddu, G.P. Littarru. (2002). Lipophilic antioxidants in human sebum and aging. Free radical research. 36(4): 471-477.

[16] G.S. Kelly. (1999). Squalene and its potential clinical uses. Alternative medicine review: a journal of clinical therapeutic. 4(1): 29-36.

[17] L.L. Gershbein, E.J. Singh. (1969). Hydrocarbons of dogfish and cod livers and herring oil. Journal of the American Oil Chemists' Society. 46(10): 554-557.