ISOLATION AND CHARACTERIZATION OF TRITERPENOID AND TWO PHYTOSTEROLS FROM THE ROOT OF *FICUS ITEOPHYLLA* LINN (MORACEAE)

ABDULMALIK, I.A.[1,2*], HASSAN, H.S.[2], SULE, M.I.[2], MUSA, A.M.[2], ABDULLAHI, M.I.[3] AND MAGAJI, M.G.[4]

[1]Department of Applied Chemistry, College of Science and Technology Kaduna Polytechnic, Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, [4]Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Pharmaceutical and Medicinal Chemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.

ABSTRACT

This study reports the isolation and characterization of some bioactive compounds from the root of *Ficus iteophylla*. Powdered root of the plant was extracted with hexane and was subjected to chromatographic separation over a silica gel G (50–200) and preparative thin layer chromatography. Three compounds were isolated and coded as AMX, AM and F4. In ^{1}H-NMR of AMX, H-3 proton appeared as a doublet of doublets at δ_H 3.20 ppm, two broad singlets at δ_H4.57 ppm and 4.69 ppm, seven methyl singles at δ_H 0.78 ppm, 0.85 ppm, 0.95 ppm, 0.98 ppm, 1.01 ppm, 1.21 ppm, 1.69 ppm and ^{13}C-NMR revealed 30 signals typical for lupane skeleton. The AM ^{1}H-NMR showed H-3 proton as multiplets at δ_H 3.50 ppm, H-6 as singlet at δ_H 5.36 ppm, six methyl protons appeared as two three protons singlets at δ_H 1.01 ppm and 1.25 ppm, one three protons broad singlet at δ_H 1.59 ppm, two doublets for three protons at δ_H 0.69 ppm and 0.92 ppm, one doublet of triplets for three protons at δ_H 0.83 ppm and ^{13}C-NMR revealed the signals for all the twenty-nine carbon atoms. The ^{1}H-NMR of F4 revealed six methyl protons as two methyl singlets at δ_H 0.70 ppm and 1.08 ppm, two methyl doublets at δ_H 0.85 ppm and 1.02 ppm, two triplets at δ_H 1.16 ppm and 0.80 ppm, the H-3 proton appeared as multiplets at δ_H 3.52 ppm, three olefinic protons at δ_H 5.35 ppm (d), 5.02 ppm (dd), 5.15 ppm (dd) and the ^{13}C-NMR revealed twenty nine carbon signals. From physical, chemical and spectral characterization of AMX, AM and F4 were concluded as Lup-20(29)-ene-3-ol, Stigmat-5-ene-3-ol and Stigmat-5, 22-diene-3-ol respectively.

Keywords: *Ficus iteophylla*, Lup-20(29)-ene-3-ol, Stigmat-5-ene-3-ol, Stigmat-5, 22-diene-3-ol, ^{1}H-NMR, ^{13}C-NMR
***Correspondence:** aaamalik49@gmail.com, 08069451993

INTRODUCTION

The search for eternal health, longevity and remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings which led to the use of many plants, animal products and minerals for the development of a variety of therapeutic agents. Throughout the ages, humans have relied on plants for their basic needs for the production of medicines, food-stuffs, shelters, clothing and means of transportation [1]. Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. Traditional medicine is viewed as a combination of knowledge and practice used in diagnosing, preventing, and eliminating disease. This may rely on past experience and observations handed down from generation to generation either verbally, frequently in the form of stories, or spiritually by ancestors or, in modern times, in writing [2].

Medicinal plants are plants in which one or more of its organs/parts contain substances that can be used for therapeutic purposes, or in a more modern concept, the constituents can be used as precursors for the synthesis of drugs.

Ficus iteophylla is a monoecious, deciduous or large shrub, growing to a height of 6.9–12 metres high and found in countries of the Sudan Savanna forest and Sahel, with smooth grey bark. Its fragrant leaves are 12–20 cm long and 10–18 cm across, and deeply lobed with three or five lobes. The plant is locally called Shirinya (Hausa); Odan (Yoruba); Jeja (Kanuri); Kinde (Tiv); Jammeiz al Abiad (Shuwa Arabic) [3].

The bark is said to be used against dysentery, and externally to embrocate on areas of rheumatic pain. The powdered bark is use as a dressing on cuts and wounds. Fresh bark has veterinary use, pounded and moistened for application to horses' swollen feet. The root, leaf and bark have wide usage for treating paralysis, tuberculosis, insanity, epilepsies, convulsions, spasm, bacteria, fungi and pulmonary troubles [4].

In this study, we report on the isolation and characterization of a pentacyclic triterpenoid (3-hydroxyl-lup 20(29)-ene) and tetracyclic unsaturated phytosterols (stigmata-5-ene-3-ol and stigmata-5, 22-diene-3-ol) (Figure 1) from the root of *Ficus iteophylla*.

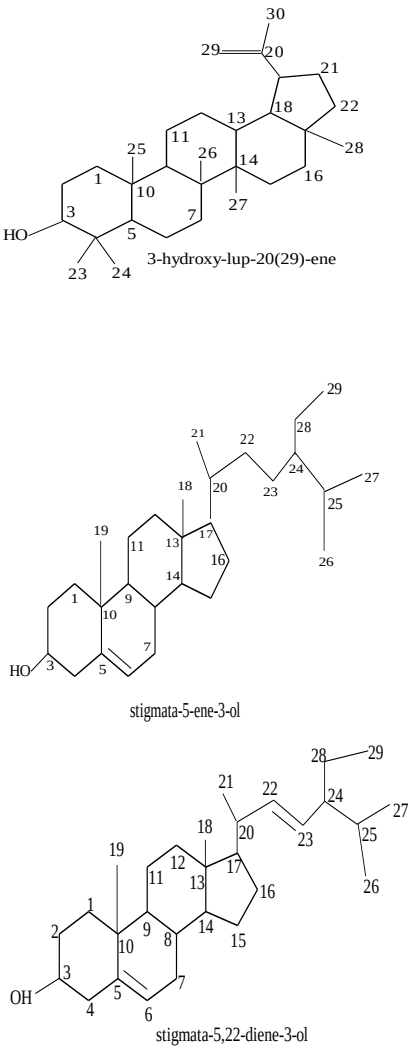

Figure 1: Structures of 3-hydroxy-lup 20(29)-ene, stigmata-5-ene-3-ol and stigmata-5, 22-diene-3-ol.

MATERIALS AND METHODS

General procedures

Gallenkemp electro thermal melting point apparatus available at Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria-Nigeria. Bruker Avanche-300 (500 and 600 MHz) Nuclear Magnetic Resonance Spectrometer available at School of Chemistry, Universiti Teknologi Malaysia (UTM) Malaysia. All organic solvents/reagents used are of analytical grade and were distilled before use. Silical gel (50–200) mesh was used for column chromatographic separation. Thin layer chromatography used contain silica gel 60 F254 of dimension20 cm x 20 cm with layer thickness of 0.25 mm.

Collection and identification of the plant material

The root of *F. iteophylla* was collected from Dajin Kudingi at Ahmadu Bello University, Zaria. The identity of the plant was confirmed and authenticated at the Botany Department, Ahmadu Bello University, Zaria comparing it with the existing specimen of voucher number 7167.

Extraction procedure and fractionation

The powdered root (850 g) of the plant was exhaustively extracted with hexane using soxhlet apparatus and concentrated to afford hexane root extract (HRE). The marc resulted from hexane root extract was air dried, re-extracted with methanol to afford methanol root extract (MRE).

Column chromatographic separations

The hexane root extract (5 g) was chromatographed over silica gel (50-200 mesh) packed column of dimension 75 by 2.8 cm, the column was eluted continuously beginning with hexane 100%, followed by hexane : chloroform mixture (95 : 5) then hexane : ethylacetate mixture in the following ratio of increasing polarity 95 : 5, 75 : 5 and 50 : 5. Thirty two fractions of 60 ml, 30 ml, 20 ml, 10 ml and 5 ml each were collected. The fractions with similar TLC profile were pooled together. This afforded eight major fractions coded P – X, where fractions V and X were pooled together based on their TLC profile and subjected to preparative thin layer chromatography that led to isolation of two compounds coded **AMX** and **AM**. The major fraction U was further subjected to column chromatography with dimension 41.0 cm by 1.0 cm eluted with hexane:ethylacetate mixture (7.5 : 0.5)which led to isolation of compound coded **F4**.

Spectroscopic characterization

The spectroscopic methods were used to elucidate the structure of isolated compounds. These include ^{1}H-NMR, ^{13}C-NMR, DEPT, 2D-NMR and FT-IR. The melting points were determined on a Gallenkemp electrothermal melting point apparatus and are uncorrected available at Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria. Thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC) were used using silica gel 60 F_{254} pre-coated plates by MERCK of thickness 0.25 mm and the column was packed with silica gel (50–200mesh). Salkowski and Liebermann-Burchard test was used as chemical test for steroids and triterpenoid.

RESULTS

The results of chemical test using Salkowski and Liebermann – Burchard indicated positive on AMX,

AM and F4 (Figure 1) they were taken to be a triterpenoid and sterols. The melting point of AMX was in the range 213ºC – 215ºC, AM in the range 136ºC – 138ºC and F4 in the range 175ºC – 176ºC this corresponds to the molecular formula $C_{30}H_{50}O$, $C_{29}H_{50}O$ and $C_{29}H_{48}O$ respectively.

Compound AMX: white powder, mp 213-215 ºC, IR (cm^{-1}): 3384, 2916, 2851, 1654, 1192, 1033, 826, 692. ^{1}H-NMR ($CDCl_3$, 600 MHz) and ^{13}C-NMR (in $CDCl_3$) with data from literature are tabulated in Table 1.

Compound AM: white powder, mp 136-138 ºC, IR (cm^{-1}): 3340, 2922, 2851, 1735, 1664, 1461, 1374, 1330. ^{1}H-NMR, ($CDCl_3$, 600 MHz) and ^{13}C-NMR (in $CDCl_3$) with data from literature are tabulated in Table 2.

Compound F4: white powder, mp 175-176 ºC, IR (cm^{-1}): 3354, 2934, 2860, 1667, 1453, 1377, 1363. ^{1}H-NMR, ($CDCl_3$, 600 MHz) and ^{13}C-NMR (in $CDCl_3$) with data from literature are tabulated in Table 3.

Table 1: Comparison of ^{1}H-NMR and ^{13}C-NMR of compoud AMX with that of 3-hydroxy-lup-20(29)-3-ene data reported in the literature.

	Data from literature (Abdullahi *et al*., 2013)		**Isolated Compound (AMX)**	
Carbon	δ_H (mult, J Hz)	δ_c	δ_H (mult, J Hz)	δ_c
1		38.7		38.7
2	1.60	27.4	1.60	27.4
3	3.2(1H,m,H3)	79.0	3.2(1H,m,H3)	79.0
4		38.9		38.9
5		55.5		55.3
6		18.5		18.3
7		34.2		34.3
8		40.9		40.9
9		50.5		50.5
10		37.2		37.2
11		21.0		21.0
12		25.2		25.2
13		38.1		38.1
14		42.9		42.9
15		27.1		27.4
16		35.5		35.6
17		43.0		43.0
18	1.89	48.3	1.89	48.4
19	2.37	48.0	2.37	48.0
20		151.0		151.0
21	1.37	29.9	1.40	29.9
22		40.0		40.0
23	0.77	28.0	0.98	28.0
24	0.97	15.5	1.21	15.4
25	0.85	16.1	0.85	16.1
26	0.94	16.0	1.01	16.0
27	1.05	14.8	0.95	14.6
28	0.79	18.0	0.78	18.0
29	4.70,4.55(s,2H)	109.0	4.57,4.69(s,2H)	109.3
30	1.68	19.5	1.69	19.3

Table 2: Comparison of ^{1}H-NMR and ^{13}C-NMR of compoud AM with that of stigmata-5-ene-3-ol data reported in the literature.

	Data from literature (Roandi *et al*., 2017)		**Isolated Compound (AM)**	
Carbon	δ_H (mult, J Hz)	δ_c	δ_H (mult, J Hz)	δ_c
1		37.2		37.23
2		32.0	1.50	31.90
3	3.51	71.8	3.50 (m, 1H)	71.83
4		42.2	2.25	42.28
5		140.7		140.78
6	5.37	121.7	5.36 (br s, 1H)	121.71
7		31.6	1.98	31.64
8		31.9	1.70	31.90
9		50.1		50.09
10		36.5		36.49
11		21.0		21.06
12		39.7		39.74
13		42.3		36.13
14		56.7		56.74
15		26.0		25.95
16		28.9		28.24
17		56.0		56.01
18	0.73 (s, 3H)	11.8	1.01 (s, 3H)	11.85
19	1.18 (s, 3H)	19.0	1.25 (s, 3H)	19.01
20		40.5		29..70
21	1.02 (d, 3H, J=7.5Hz)	18.7	0.82 (td,3H,J=12,6,6Hz)	18.77
22		33.9		33.91
23		36.1		25.95
24		51.2		45.79
25		29.1		29.14
26	0.80 (d, 3H, J=6.0Hz)	19.8	0.69 (d,3H,J=6Hz)	19.82
27	0.85 (d, 3H, J=6.0Hz)	19.4	0.92(d,3H,J=6Hz)	19.40
28		23.0		23.03
29	0.81 (m, 3H)	11.9	1.52 (m, 3H)	11.98

Table 3: Comparison of ^{1}H-NMR and ^{13}C-NMR of compoud F4 with that of stigmata-5, 22-diene-3-ol data reported in the literature.

	Data from literature (Chaturvedula and Prakash, 2012)		**Isolated Compound (F4)**	
Carbon	δ_H (mult, J Hz)	δ_c	δ_H (mult, J Hz)	δ_c
1		37.6		37.25
2		32.1		31.63
3	3.51(tdd,1H,J=4.5,4.2,3.8Hz)	72.1	3.52 (m, 1H)	71.79
4		42.4		42.28
5		141.1		140.74
6	5.31(t,1H,J=6.1Hz)	121.8	5.35 (d, 1H,J=5Hz)	121.70
7		31.8		28.93
8		31.8		31.89
9		50.2		50.14

10		36.6		36.50
11		21.5		21.06
12		39.9		39.67
13		42.4		42.20
14		56.8		55.94
15		24.4		24.36
16		29.3		28.93
17		56.2		56.86
18	0.71 (s, 3H)	18.9	0.70 (s, 3H)	12.26
19	1.03 (s, 3H)	12.2	1.25 (s, 3H)	19.39
20		40.6		40.51
21	0.91(d, J=6.2, 3H)	21.7	0.85 (d, 3H, J=5Hz)	21.10
22	4.98(m, 1H)	138.7	5.02 (dd,1H,J=5, 5Hz)	138.33
23	5.14(m,1H)	129.6	5.15 (dd, 3H,J=10,10Hz)	129.26
24		46.1		51.24
25		29.6		31.89
26	0.80 (d, 3H, J=6.6 Hz)	19.8	1.02 (d, 3H,J=10Hz)	21.22
27	0.82 (d, 3H, J=6.6 Hz)	20.2	1.16 (t, 3H.J=10Hz)	18.98
28		25.4		25.41
29	0.83 (t, 3H, J=7.1 Hz)	12.1	0.80 (t, 3H,J=7.5Hz)	12.05

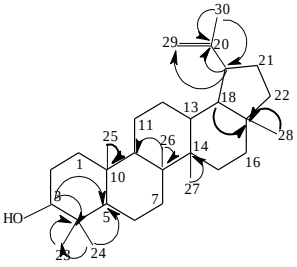

Figure 2: HMBC correlation of 3-hydroxy-lup 20(29)-ene

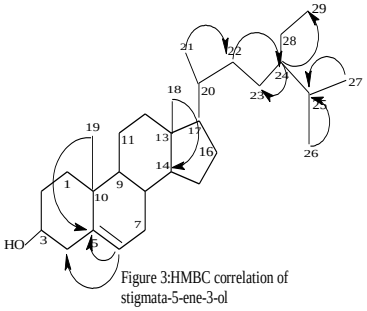

Figure 3:HMBC correlation of stigmata-5-ene-3-ol

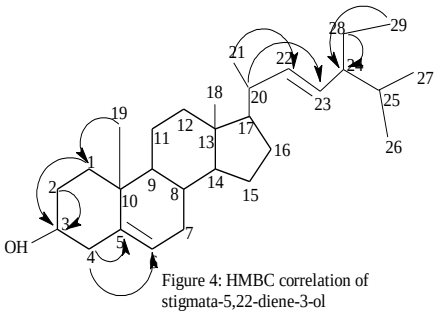

Figure 4: HMBC correlation of stigmata-5,22-diene-3-ol

DISCUSSION

The ^{1}H-NMR spectrum (600MHz, $CDCl_3$) of AMX showed two broad singlets at δ_H 4.57 ppm and δ_H 4.69 ppm which suggest two exomethylene (olefinic) protons attached to C-29 a chacteristic feature of lupeol [5]. A doublet of doublet at δ_H 3.20 ppm was due to the methine proton attached to the secondary carbinol at C-3 which further confirmed that AMX is a triterpenoids [6]. It also revealed seven methyl singlets at δ_H 0.78 ppm, δ_H 0.85 ppm, δ_H 0.95 ppm, δ_H 0.98 ppm, δ_H 1.01 ppm, δ_H 1.21 ppm and δ_H 1.69 ppm [5], the signal at δ_H 7.25 ppm was due to deuterated chloroform ($CDCl_3$) used. The ^{13}C-NMR revealed 30 signals typical for the terpenoid of lupane skeleton which includes the olefinic carbons of the exocyclic double bond which appeared at δc 150.97 ppm (C-20) andδc 109.31 ppm (C-29). The DEPT (135, 90 and 45) spectra established the multiplicity of each carbon, it acts as a way to encode information about the number of protons attached to a carbon. DEPT 135 of AMX showed 13 signals for Sp^1 and Sp^3 carbon (Up) while 11 signals represent Sp^2 carbon (Down) and 6 quaternary carbons. From DEPT spectra it was deduced that the molecular formula is $C_{30}H_{50}O$. The COSY spectrum of AMX revealed that there is a correlation between signals at δ_H 3.20 ppm (H-3) and δ_H 1.55 ppm (H-2); δ_H 2.35 ppm (H-19) and δ_H 1.30 ppm (H-21); δ_H 1.88 ppm (H-18) and δ_H 130 ppm (H-21). The HSQC spectrum showed the attachments of all protons in AMX to their respective carbons, for examples δ_H 4.57 ppm (H-29) and δ_H 4.69 ppm (H-29) were attached to carbon 29 at δ_c 109.31 ppm, the methine proton at δ_H 3.20 (H-3) attached to C-3 δ_c 79.03. The structure was further supported by key HMBC correlation as shown in figure 2. The IR of 3-hydroxy-lup-3-ene showed a broad band at 3384 cm^{-1} for hydroxyl group. The methyl stretching

and bending vibration occurred at 2916 cm^{-1}. The band at 2851 cm^{-1} indicated the vibration of methylenic part. The C-C vibration occurred at 1033 cm^{-1}. The band at 1192 cm^{-1} and 672 cm^{-1} were observed for the OH bond vibration of the hydroxyl group. The C-H vibration of the unsaturated was observed at 826 cm^{-1}while C=C vibration occurred at 1654 cm^{-1}. Based on the above spectral analysis, AMX was confirmed to be 3-hydroxy-lup-3-ene. All the data were in agreement with 3-hydroxy-lup-3-ene reported in literature [7, 8].

The expanded ^{1}H-NMR spectrum ($CDCl_3$, 600MHz) of AM showed two three-proton singlets at δ_H 1.01 ppm (3H, s, H-18) and δ_H 1.25 ppm (3H, s, H-19) due to the C-18 and C-19 methyl protons respectively. One broad singlet at δ_H 1.59 ppm (3H, br s. H-29) was due to C-29 methyl protons. Two doublets, integrating for three protons, at δ_H 0.69 ppm (3H, d, J = 6.0 Hz, H-26), δ_H 0.92 ppm (3H, d, J = 6.0 Hz, H-27) were ascribed to the C-26, C-27 methyl protons respectively while signal at δ_H 0.83 ppm (3H, dt, J = 12, 6, 6 Hz, H-21) was ascribed to C-21. The resonance of methine proton CH-OH at δ_H 3.50 ppm (1H, m, H-3) an indication for the presence of OH group at C-3, the olefinic proton resonated at δ_H 5.36 ppm (1H, s, H-6) an indication of C=CH between C-5 and C-6. The signal resonated at δ_H 7.25 ppm was due to the solvent $CDCl_3$ used with some impurities surrounding the signal.

The ^{13}C-NMR spectrum ($CDCl_3$, 600MHz) of the AM showed the resonance of all twenty nine carbon atoms. The DEPT 135 spectrum of AM showed the multiplicity of each carbon signal in the broadband ^{13}C-NMR spectrum and it revealed the presence of six methyl, eleven methylene, nine methine carbons and three quaternary carbons. The C-3 methine proton δ_H 3.50 showed vicinal couplings (^{3}J correlation) with the C-2 methylene (δ_H1.50 ppm) and C-4 methylene (δ_H 2.25 ppm) also vicinal coupling occurred between the olefinic proton (H-6) at δ_H 5.36 ppm with methylene protons of C-7 at δ_H 1.98 ppm. The HSQC spectrum of the AM was used to assign protons to their respective carbons. Proton at position δ_H 5.36 ppm is on carbon position δ_c 121.71 ppm, that at δ_H 3.50 ppm on carbon δ_c 71.83 ppm, that at δ_H 2.25 ppm on carbon δ_c 42.31 ppm and that at δ_H 1.99 ppm on carbon δ_c 31.91 ppm.

The HMBC showed interactions of compound AM as shown in figure 3. The IR spectrum showed broad absorption peak at 3340 cm^{-1} indicating the presence of –OH group. The peak at 2922cm^{-1} and 2851 cm^{-1} indicated the presence of saturated aliphatic C-H stretching. The unsaturated C=C absorption peak occurred at1735 cm^{-1} and 1664 cm^{-1}. The absorption band at 1461 cm^{-1} indicated the presence of C-H bending of CH_2 but the absorption band at 1374 cm^{-1} and 1330 cm^{-1} is due to C-H bending of gem-dimethyl groups. The ^{1}H-NMR, ^{13}CNMR, DEPT and 2D-NMR data of AM were all in agreement with the data reported in the literature of stigmata-5-ene-3-ol or sitosterol or cholesta-5-ene-24-ethyl-3-ol [9, 10]. Based on these spectral data the AM was established as stigmat-5-ene-3-ol or cholest-5-ene-24-ethyl-3-ol or β-sitosterol.

The expanded ^{1}H-NMR spectrum ($CDCl_3$, 500MHz) of F4 showed the presence of two methyl singlets δ_H 0.70 ppm (3H, s, H-18), δ_H 1.08 ppm (3H, s, H-19), two methyl doublets δ_H 0.85 ppm (3H,d, J = 6.2 Hz, H-21), δ_H 1.02 ppm (3H, d, J = 9.2 Hz, H-26)), two triplets δ_H 1.16 ppm (3H, t, J=9.5 Hz, H-26) and δ_H 0.80 ppm (3H, t, J=7.5 Hz, H-29). The methine proton at C3 appeared as multiplets at δ_H 3.52 ppm (1H, m, H-3). Three olefinic protons appeared at δ_H 5.35 ppm (1H, d, J = 4.75 Hz, H-6), δ_H 5.02 ppm (1H, dd, J = 8.7, 8.7 Hz, H-22) and δ_H 5.15 ppm (1H, dd, J = 8.7, 8.7 Hz, H-23). The ^{13}C-NMR spectrum showed 29 carbon signals including recongnizable signals at δc 140.7 ppm (C5), δc 121.7 ppm (C6), δc 138.33 ppm (C22), δc 129.26 ppm (23). The DEPT 135 revealed that F4 has six methyl carbon, nine methylenes carbon, eleven methine and three quaternary carbons. In the cosy spectrum of F4 there was a vicinal coupling at δ_H 3.52 ppm (H-3) with δ_H 2.25 ppm (H-4) andδ_H 5.35 ppm (H-6) with δ_H 1.88 ppm (H-7).

The hetronuclear single quantum correlation (HSQC) of F4 was used to assign all protons to their respective carbons for example δ_H 5.31 ppm (H-6) was assigned to δc 121.70 ppm (C-6), δ_H 3.51 ppm (H-3) was assigned to δc 72.1 ppm (C-3), δ_H 4.98 ppm (H-22) was assigned to δc 138.33 ppm (C-22), δ_H 5.15 ppm (H-23) was assigned to δc 129.26 ppm (C-23) while HMBC correlation was shown in figure 4. The IR spectrum of stigmasterol exhibited the band of a hydroxyl group at 3354cm-1 and two sharped absorption at 2934 cm^{-1} and 2860 cm^{-1} for C-H stretching for sp^3 hybridized aliphatic carbon. The spectrum also revealed absorption at1667 cm^{-1} for C=C stretching sp^2 hybridized. The band at 1453 cm^{-1} is for C-H bending CH_2 group while that at 1377 cm^{-1} and 1363 cm^{-1} is due to CH bending of gem-dimethyl groups. The^{1}H-NMR, ^{13}C NMR, DEPT, COSY, HSQC and HMBC values of F4 were in agreement with that reported for stigmata 5, 22-diene-3-ol or cholesta 5,22-diene-24 ethyl -3-ol or stigmasterol in the literature [11 - 14]. Based on the above data analyses the compound F4 was named stigmat 5, 22-diene-3-ol.

CONCLUSION

One pentacyclic lupane triterpenes and two tetracyclic unsaturated phytosteroids were isolated from hexane root extract of *Ficus iteophylla.* The structures of these compounds were identified as lup-20(29)-ene-3-ol

(AMX), stigmat-5-ene-3-ol (AM) and stigmat 5, 22-diene-3-ol (F4) on the basis of their spectroscopic, comparing their physical properties and spectral data to those reported in the literature respectively.

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