American Journal of Organic Chemistry 2012, 2(2): 14-20
DOI: 10.5923/j.ajoc.20120202.03

Isolation and Elucidation of Three Triterpenoids and Its Antimycobacterial Activity of Terminalia Avicennioides

Abdullahi Mann[1,*], Kolo Ibrahim[2], Adebayo O. Oyewale[3], Joseph O. Amupitan[3], Majekodumi O. Fatope[4], Joseph I. Okogun[5]

[1]Department of Chemistry, Federal University Technology, Minna, P. M. B. 65, Niger State, Nigeria
[2]Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD), P.M.B.21, Garki – Abuja, Nigeria
[3]Department of Chemistry, Ahmadu Bello University Zaria, Kaduna State, Nigeria
[4]Department of Chemistry, College of Science, Sultan Qaboos University, P.O. Box 36, Postal Code 123, Al-Khod, Muscat, Oman
[5]Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), P.M.B. 21, Garki – Abuja, Nigeria

Abstract Terminalia avicennioides Guill. & Perr. (Combretaceae) has been traditionally used as traditional medicine for centuries in Nupeland, North Central Nigeria for the treatment of respiratory diseases such as tuberculosis and cough. This study evaluates the in vitro antimycobacterial activities of the isolated compounds in order to support the therapeutic use of T. avicennioides for treating these infectious diseases. Chemical investigation by bioassay-guided fractionation led to the isolation of three triterpenoids namely, arjunolic acid (1), α-amyrin (2) and 2, 3, 23-trihydroxylolean-12-ene (3). Among them, arjunolic acid manifested the most potent antimycobacterial activity against a strain of Mycobacterium bovis (BCG). Structure elucidation of the isolated compounds were based primarily on the analysis of 1-D and 2-D NMR spectral data including HMQC, HMBC, COSY and NOESY correlations, as well as comparison with reported authentic data of arjunolic acid, α-amyrin and 2, 3, 23-trihydroxylolean-12-ene. To the best of our knowledge, this is the first time these compounds (1-3) are reported from this plant. The present result further confirms the value of ethnopharmacological investigations into traditional herbs for leads for potential drug development.

Keywords Antimicrobial Activity, Terminalia Avicennioides, Triterpenoids

1. Introduction

Antimicrobial agents are chemical or biological substances used to kill or prevent the growth of microorganisms. A great number of these agents already exist and their actions on microorganisms are due to the presence of certain substances in plants. Plants are known to have special ability to synthesize aromatic substances, most of which are phenols or their derivatives [1]. Though some of these compounds may not have any discernible physiological roles in the plants in which they occur, many of them have significant biological effects on animals. In fact, they are responsible for the therapeutic effect of medicinal plants. Some of these metabolites have been isolated and found in vitro to have antimicrobial properties [2]. Some of the chemical substances are nutritious, poisonous, hallucinogenic or thera peutic in nature. Generally, plants have been described as the sleeping giants of pharmaceutical industries for the provision of much-needed antimicrobial agents [3]. Some of the same herbs and spices used by humans in our daily dishes to season food yield useful medicinal compounds [4].

Terminalia avicennioides Guill & Perr (Combretaceae) grow as shrubs in savanna region in Nigeria [5]. The shrub is known as 'kpace' in Nupe, 'kpayi' in Gwari, and 'baushe' in Hausa [6]. From the literature information this plant is reputed for several medicinal uses in Nigeria [7-10]. In recent times, there have been many preliminary phytochemical investigations of this plant to explore and exploit its pharmacological potentials. Since this plant is very common in Nigeria, further study and investigation of its active constituents may prove its potential biological activities and medicinal values. Compounds with antimycobacterial activities have been isolated from plants [11]. Some compounds earlier isolated from T. avicennioides are hydrolysable tannins that indicated antitrypanosomal activity [12]. However, extensive array of triterpenes has been reported to exhibit diverse biological activities such as anti-HIV-1 activity [13], antimycobacterial activity [11], antifungal and antimicrobial [14,15]; antiulcerogenic, anti-inflammatory, fibrocystic, antipyretic, analgesic, larvicidal, and antiedematous activities [16,17]. Moreover, information on triterpenoidal compounds of T. avicennioides

* Corresponding author:
abdumann@yahoo.com (Abdullahi Mann)
Published online at http://journal.sapub.org/ajoc

is lacking. We therefore investigate the antimycobacterial activity of the root bark extract of T. avicennioides used in Nupeland, North Central Nigeria for the management of tuberculosis which is an opportunistic infection among the HIV/AIDS patients.

2. Materials and Methods

2.1. Plant Material

The root bark of Terminalia avicennioides (Nupe: Kpace) were obtained from their natural habitats in a forest near Emitete, Lavun Local Government, Niger State, Nigeria in August, 2008. This plant was identified and authenticated by Mallam Ibrahim Muazzami of the Department of Medicinal Plant Research and Traditional Medicine of National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Abuja, Nigeria where a voucher specimen (NIPRDH 5735) was deposited at the herbarium unit of this same institution.

2.2. Extraction, Fractionation and Isolation

Air-dried ground root bark of Terminalia avicennioides (5 kg) was macerated successively with n-hexane (n-Hex), ethylacetate (EtOAc), chloroform ($CHCl_3$) and methanol (MeOH) (2 x 2.5 L each) at room temperature for 72 h. The various solvent soluble extracts obtained from the sequential extractions were recovered from the marc and concentrated (in vacuo) under reduced pressure at 35℃ using rotary evaporator (Büchi Rotavapor R-205). The marc was then discarded. The air-dried solvent extracts obtained were packed in well labelled vials, wrapped with aluminium foil and kept in the fridge until required. Phytochemical screenings were performed as described in previous works [11] (Mann et al., 2008c; 2009). n-Hex and EtOAc soluble extracts were subjected to column chromatography with glass column (Φ2.0 x 40 cm) packed with slurry of silica gel (70 – 230 mesh, 40g) (Merck, Darmstadt, Germany) and then eluted successively with gradient system of n-Hex-EtOAc (100:0→95:5→90:10→80:20→40:60→ 50:50→40:60→20:80→10:90→5:95→0:100). The eluents were collected and combined based on their Rf values obtained from the TLC behaviour.

2.3. Phytochemical Screening

Phytochemical tests were conducted on n-hexane fraction and three isolated compounds of T. avicennioides to determine the presence of alkaloids, tannins, terpenoids, saponins, anthraquinones and carbohydrates using standard protocols [18-20].

2.4. Mycobacterium Bovis (BCG) and Inocula Preparation

BCG was obtained from National Institute of Allergic Diseases (NIAD), TB Research Section, NIH, Maryland, USA and cultured at Department of Microbiology and Biotechnology, NIPRD, Garki – Abuja. BCG cultured and grown on Lowenstein Jensen medium (LJ) and then subcultured in Middlebrook 7H9 broth supplemented with Albumin Dextrose Complex (ADC) at 37℃ for 14-21 days were measured on spectrophotometer to be OD 0.2 - 0.3 at 650 nm. The cultures were then diluted at $^1/_{1000}$ by adding 25 µL cell culture to 25 mL medium.

2.5. Determination of Antimycobacterial Activity

The antimycobacterial activity test was conducted on the isolated triterpenoidal compounds as earlier described [11].

3. Results and Discussion

3.1. Extraction and Fractionation

All soluble extracts were concentrated and dried in vacuo gave extractive values as indicated in the earlier report [11]. The n-hexane extract (3 g, oily) subjected to successively elution with gradient system gave nine fractions namingly: TaF1 (F1-9), TaF10 (F10-16), TaF17 (F17-19), TaF20 (F20-30), TaF31 (F31-46), TaF47 (F47-77), TaF79 (F79-96), TaF97 (F97-110) and TaF111 (F111-126). These fractions were labelled based on their TLC behaviour. Further chromatographic fractionation of the combined subfractions gave two prominent compounds (TAP40 and TAP28). While column chromatographic separation of a fresh n-hexane extract gave C1-C73 fractions which on further purification yielded TAPE51 as major constituent. In the present report we attempt to elucidate the structures of TAP28, TAP40 and TAPE51.

3.2. Phytochemical Analysis of Extract

The results of the extract of phytochemical analysis prominently indicate the presence of saponins, steroids, tannins, terpenes and carbohydrates as shown in Table 1. All the three elucidated compounds were positive with Liebermann-Burchard's test which is indicative of triterpenoid (Table 1).

Table 1. Phytochemical screening results of the n-hexane fraction and three isolated compounds

Plant species	A	An	Cb	F	S	St	T	Tp
T. avicennioides	-	+	-	-	+	+	+	+
Compound 1 (TAP40)	-	-	-	-	-	-	-	+
Compound 2 (TAP28)	-	-	-	-	-	-	-	+
Compound 3 (TAPE51)	-	-	-	-	-	-	-	+

Key: (+)→ present, (-)→ absent, A→Alkaloids, An→ Anthraquinone, Cb→ Carbohydrate, F→Flavonoid, S→Saponin, St→Steroids, T→Tannin, Tp→Terpenoids.

3.3. Structural Elucidation of Compound 1 (TAP40)

Compound 1 (TAP40) was obtained as a white amorphous solid, mp 231-232℃ (decomp); $[\alpha]_D^{20}$ +2° (c.1.0, MeOH) and TLC Rf value of 0.41 (EtOAc–MeOH–AcOH, 9:1: one drop). The IR spectrum showed absorption at 3422 and 1695 cm^{-1} suggestive of the presence of hydroxyl and carbonyl groups. The ^{13}CNMR (DEPT) spectra suggests the presence

of 30 carbons made of seven methyls, nine methylenes (including one oxygen-bearing (δ 67.53), six methines (including one olefinic (δ 124.58) and two oxygen-bearing (δ 69.24 and 78.89) ones and eight quaternary carbons (including one carboxyl (δ 179.94), and one olefin (δ 144.83) (Table 2). The ^{1}H-NMR signals at δ 0.74 (s, 3H, Me-24), 0.77 (s, 3H, Me-25), 0.92(s, 3H, Me-26), 0.99 (s, 3H, Me-29), 0.96 (s, 3H, Me-30), and a broad signal at 5.33 (H-12) together with the ^{13}C-NMR suggests the presence of olean-12-ene skeleton [21]. The ^{1}H-NMR showed a doublet of a triplet at 3.64 (J = 7 Hz, J = 10 Hz), and at doublet, δ 4.04 (J = 11Hz) which were assigned to H-2 and H-3. Two AB signals at δ 3.38 and 3.35 suggests the presence of a –CH_2OH function attached to a quaternary carbon and was assigned to C-23. In addition, the chemical shifts of C-4 and C-24 led to placement of the -CH_2OH at the C-23 position. In the HMBC correlation there was connectivity between Me-25/C-1, 5, 9, 10; Me-26/C-7, 8, 9, 14; Me-23/C-3, 4, 5, 24; Me-29/C-19, 20, 21, 30; Me-30/C-19, 20, 21, 29. Taken together, compound 1 was identified as arjunolic acid by spectral comparison with published data for arjunolic acid [21]. The structure is established as shown in figure 1.

3.4. Structure Elucidation of Compound 2 (TAPE51)

Compound **2** (TAPE51) was obtained as a colorless needles, mp 195-196℃.); $[\alpha]_D^{20}$ −3° (c.0.43, $CHCl_3$) and TLC Rf value of 0.35 (EtOAc–MeOH–AcOH, 9:1: one drop). It responded positively to the Liebermann-Burchard test for pentacyclic triterpene. The IR spectrum of **2** showed characteristic absorption bands at 3282 ascribable to hydroxyl. ^{13}C- and DEPT 135° NMR (Table 3) spectra showed six signals for methyl carbons, ten methylenes, seven methines, and six quaternary carbons. A total of 30 carbon resonances were observed, which confirmed its triterpenic nature. The ^{1}H NMR spectrum displayed signals for eight tertiary methyls at δ 0.74 (3H, s, Me-25), 0.80 (3H, s, Me-28), 0.88 (6H, s, Me-29, Me-30), 0.94 (3H, s, Me-24), 0.90 (3H, s, Me-26), 1.30 (3H, s, Me-23), 1.01 (3H, s, Me-27) with one proton multiplet at δ 5.24 (1H, dd, J = 9.0, 2.0 Hz, H-3) assignable to the carbinol proton and one vinylic proton at δ 5.30 (1H, t, J = 1.0 Hz, H-12). The presence of these functionalities in the triterpenoid molecule received support from the appearance of the chemical shifts in the ^{13}C-NMR spectrum at 121.57 & 142.37 (>C=C<). Assignment of the hydroxymethine carbon C-3 (δc 71.69) was established on the basis of connectivities observed from an HMBC experiment (Table 3). Accordingly, cross-peak correlations between the carbon signals of C-23 and C-24 and H-3 resonance at δ 5.24, which in turn displayed one-bond ^{1}H-^{13}C connectivity with the carbon signal at δ 71.69 allowed the assignments of C-3/H-3. Further evidence for the structure of 2 was provided by additional two- and three-bond correlations discernible in the HMBC spectrum (Table 3). Thus, the molecular formula of compound **2** was established as $C_{30}H_{50}O$ and eventually characterized as α-amyrin in figure 1.

Table 2. ^{1}H- and ^{13}C-NMR spectroscopic data of the isolated compound 1 in $CDCl_3$

C	TAP40 (compound 1)					δc†
	δc	^{13}C-type	δ_H	Coupling const.(Hz)	H→C	
1	47.56	CH_2	1.63, 1.39	d, 10.0, 1 0.0	H-25	47.1
2	69.24	CH	3.64	td, m	–	68.2
3	78.89	CH	4.04	d, 11.0	H-23, H-24	78.7
4	43.81	C	–	–	H-2, H-23, H-24	43.5
5	49.06	CH	1.96	t, 6.0, 3.0	H-23, H-24	48.4
6	19.28	CH_2	1,75, 1.31	td, 7, 8, 17	–	18.6
7	3.82	CH_2	1.56, 1.26	t, 12, 13	H-26	33.1
8	40.76	C	–	–	H-26	40.1
9	48.75	CH	1.42	t, 10.0, 14.0	H-25, H-26	48.5
10	38.69	C	–	–	H-2, H-11, H-25	38.5
11	24.29	CH_2	3.57, 3.43	dd, 8, 11.0	–	23.8
12	124.58	CH	5.33	br s	–	123.5
13	144.83	C	–	–	H-11, H-15, H-19, H-27	144.1
14	42.76	C	–	–	H-12, H-16, H-18, H-27	42.4
15	29.49	CH_2			–	28.3
16	24.90	CH_2	3.33, 1.81	t, 7.0, 10.0	–	23.9
17	46.44	C	–	–	H-13, H-15, H-19, H-21	47.0
18	45.01	CH	2.07	t, 2.0, 2.0	H-12, H-16, H-22	43.5
19	49.96	CH_2	1.67, 1.23	d, 15.0, 15.0	H-21, H-29, H-30	46.3
20	30.69	C	–	–	H-18, H-22, H-29, H-30	30.7
21	34.01	CH_2	1.89, 1.87	t, 4, 8	–	34.2
22	33.59	CH_2	3.28, 1.70	t, 11, 15	–	33.0
23	67.53	CH_2	3.38, 3.35	s	H-3, H-4, H-5, H-24	67.2
24	14.12	CH_3	0.74	s	H-3, H-4, H-5, H-23	14.0
25	17.76	CH_3	0.77	s	H-1, H-5, H-9, H-10	17.6
26	18.13	CH_3	0.92	s	H-7, H-8, H-9, H-14	17.2
27	26.80	CH_3	1.11	s	H-8, H-13, H-14, H-15	26.1
28	179.94	C	–	–	–	178.6
29	25.39	CH_3	0.99	s	H-19, H-20, H-21, H-30	32.9
30	25.36	CH_3	0.96	s	H-19, H-20, H-21, H-29	23.7

(δ in ppm)δc †→^{13}C NMR of arjunolic acid [21; p1527

Table 3. ^{1}H- and ^{13}C-NMR spectroscopic data of the isolated compound 2 in $CDCl_3$ (δ in ppm)

C	TAPE51 (Compound 2)					δc‡
	δc	^{13}C- type	δ_H	Coupling const.(Hz)	H→C	
1	38.26	CH_2	1.50, 1.48	dd, 2.0 3.0; 3.0 2.0	H-25	38.7
2	27.79	CH	4.38, 4.19	dd, 5.0 6.0; 7.0 5.0		27.2
3	71.69	CH	5.24, 5.11	dd, 9.0 2.0; 9.0 12.0	H-23, H-24	78.3
4	43.11	C	–	–	H-2, H-23, H-24	38.7
5	57.68	CH	4.00	d, 7	H-23, H-24	55.2
6	18.25	CH_2	0.99	t, 3.0		18.3
7	32.65	CH_2	0.94	d, 11.0	H-26	32.9
8	57.78	C	–	–	H-26	40.0
9	46.75	CH	3.401	t, 3.0, 4.0	H-25, H-26	47.7
10	32.81	C	–	–	H-2, H-11, H-25	36.9
11	23.77	CH_2	2.79	t, 7.0		23.3
12	121.57	CH	5.30	t, 1.0		124.3
13	142.37	C	–	–	H-11, H-15, H-19, H-27	144.8
14	42.99	C	–	–	H-7, H-16, H-26, H-27	42.0
15	28.97	CH_2	1.78, 1.71	dd, 3.0 6.0; 2.0 6.0		28.7
16	26.80	CH_2	1.05, 1.04	dd, 6.0 8.0; 6.0 6.0		26.6
17	33.15	C	–	–	H-13, H-15, H-19, H-21	33.7
18	51.24	CH	2.26	t, 3.0, 4.0	H-12, H-16, H-22	58.9
19	43.35	CH_2	1.91, 1.90	dd, 3.0 4.0; 4.0 5.0	H-21, H-29, H-30	39.6
20	29.45	C	–	–	H-18, H-22, H-29, H-30	39.6
21	32.53	CH_2	1.22, 1.18	dd, 14.0 15.0; 5.0 15.0		31.2
22	40.69	CH_2	1.87, 1.86	dd, 5.0 2.0; 5.0 3.0		41.5
23	23.33	CH_2	1.30	s	H-3, H-4, H-5, H-24	28.1
24	23.22	CH_3	0.94	s	H-3, H-4, H-5, H-23	15.6
25	14.35	CH_3	0.74	s	H-1, H-5, H-9, H-10	15.6
26	17.10	CH_3	0.90	s	H-7, H-8, H-9, H-14	16.8
27	21.44	CH_3	1.01	s	H-8, H-13, H-14, H-15	26.0
28	21.70	CH_3	0.80	s		28.1
29	26.21	CH_3	0.88	s	H-19, H-20, H-21, H-30	17.4
30	26.10	CH_3	0.88	s	H-19, H-20, H-21, H-29	21.3

δc ‡→^{13}C NMR of α-amyrin [21; p1541]

Table 4. ^{1}H- and ^{13}C-NMR spectroscopic data of the isolated compound 3 in $CDCl_3$ (δ in ppm)

C	TAP28 (compound 3)					δc§
	δ_C	^{13}C- type	δ_H	Coupling const. (Hz)	H→C	
1	47.27	CH_2	3.80	d, 11.0	H-25	47.7
2	68.85	CH	4.09	dd, 5.0 10.0	–	68.8
3	85.86	CH	4.04	d,11.0	H-23A, H-23B	78.3
4	44.29	C	–	–	H-23, H-24	43.0
5	48.76	CH	2.36	d,10	H-23, H-25	48.8
6	19.53	CH_2	1.07	t, 3.0 4.0	–	18.5
7	33.56	CH_2	1.51	t, 13 15	H-6, H-26	33.0
8	39.28	C	–	–	H-26, H-6, H-27	39.9
9	48.76	CH	3.31	dd, 2.0 4.0	–	48.0
10	36.15	C	–	–	H-25, H-6	37.3
11	24.71	CH_2	1.40	d, 5.0	H-12	24.2
12	124.03	CH	5.32	dd, 3 3.5	H-18	123.3
13	144.82	C	–	–	H-18	144.8
14	40.77	C	–	–	H-27, H-18, H-26	41.9
15	28.38	CH_2	1.97	d, 11.0	–	28.3
16	25.12	CH_2	1.28	t, 7.0	H-18	23.3
17	34.25	C	–	–	H-18	32.5
18	44.57	CH	2.12	d, 5.0	–	44.7
19	30.53	CH_2	1.64	d, 3.0	H-29, H-30	46.8
20	30.82	C	–	–	H-29, H-30	31.0
21	33.85	CH_2	1.94	t, 7.0 6.0	H-29, H-30	34.3
22	30.05	CH_2	2.06	dd, 2.0 4.0	–	32.3
23	65.59	CH_2	4.08	s	H-3	66.0
24	14.50	CH_3	0.78	s	H-3	14.2
25	17.35	CH_3	0.85	s	–	17.2
26	17.50	CH_3	1.02	s	–	17.5
27	24.95	CH_3	1.05	s	–	26.0
28	28.51	CH_3	1.23	s	–	28.4
29	30.09	CH_3	0.99	s	H-30	33.3
30	23.73	CH_3	0.71	s	–	23.7

δc§→^{13}C-NMR of olean-12-ene data from literature [21; pp 1527 & 1529].

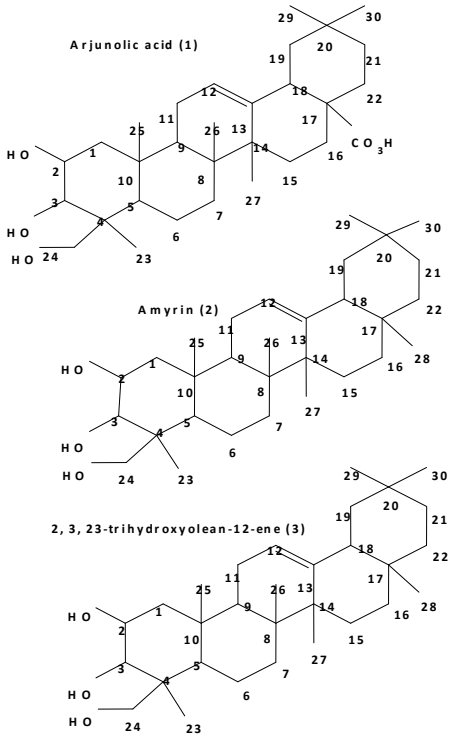

Figure 1. Triterpenoidal compounds isolated from the root bark of *Terminalia avicennioides*

3.5. Structure Elucidation of Compound 3 (TAP28)

Compound 3 (TAP28) was obtained as colourless amorphous powder with mp 271-272℃ (decomp); $[\alpha]_D^{20}$ +12° (*c*.0.43, $CHCl_3$) and TLC Rf value of 0.51 (EtOAc–MeOH– AcOH, 9:1: one drop). The IR spectrum gave absorption peaks at υ_{max} $^{(KBr)}$ cm^{-1}: 3429(OH), 2940(CH_2), 1458(CH_2 and CH_3), 1385 (gem-dimethyl), 1264 (two methyl on quaternary carbon atom) and 1035 (C-O). The ^{13}CNMR (Table 4) spectroscopic data in combination with analysis of DEPT and HSQC spectra showed the presence of 30 carbon signals due to seven quaternary, six methine, eleven methylene and six methyl carbon atoms, which were assigned to a triterpene skeleton. Careful analysis of the NMR data indicated that it is an oxygenated oleane derivative. The presence of a trisubstituted double bond was inferred by the signals of a methine carbon at δ 124.03 and a quaternary carbon at δ 144.82. A comparison between the ^{13}CNMR spectroscopic data of compound 3 with those of olean-12-ene [21] indicated the presence of three oxymethines at C-2, C-3, and C-23 and the double bond between C-12 and C-13 with an oxymethine (δc 124.03) and quaternary carbon (δc 144.82). In the HMQC spectrum a cross-peak correlation was observed between the former carbon signal and the broad hydrogen singlet at δ 5.32, which was assigned to the vinylic hydrogen. In the ^{1}H NMR spectrum, the signals at δ 4.04 (br s) and 4.09 (dd, *J* 5.0 and 10.0 Hz) which showed connectivities in the HMQC spectrum with the carbon signals at δ 68.85 and 85.86 respectively, were attributed to two carbinolic hydrogens. Similarly, the broad singlet at δH 4.08 (2H) which showed cross-peak correlation with the carbon signal at 65.59 were assigned to hydroxymethylene hydrogen. This information, along with the absorption at νmax 3429 cm^{-1} observed in the IR spectrum, led to the assumption that 3 was an olean-12-ene-type triterpene with two hydroxymethylene and one secondary hydroxyl group and its molecular formula established as $C_{30}H_{50}O_3$ in figure 1.

3.6. Antimycobacterial Activity of the Triterpenoidal Compounds against BCG

Microbial infections are great challenge to human health concern and it is even exacerbated by the growing resistance to the conventional drugs [22,23]. Thus, researchers have resort to find remedy from plants for infectious diseases. Natural products are typically secondary metabolites, produced by plants in response to external stimuli such as nutritional changes, infection and competition [24,25]. Therefore, bioactive natural products are chemical substances produced by the host as defensive and protective mechanisms against predation by microorganisms, insects and herbivores [26]. Compounds like terpenoids give plants their odours and flavour; tannins are responsible for pigment [27]. These natural products isolated from higher plants have been isolated as major source of novel biologically active pharmacophores or chemically active drugs [28]. Some reviews on antimycobacterial natural products indicate that triterpenes possess potential structural skeletons which could provide useful scaffolds or templates for the development of new antimycobacterial drugs [29-31]. This observation makes this class of compounds interesting for further investigation and special attention on the T. avicennioides. Consequent upon this and in continuation of our search for antimycobacterial activities from T. avicennioides, we fractionate the root bark of T. avicennioides in order to exploit the individual bioactive constituents responsible for the exhibited activities. Previous investigations of the root bark of T. avicennioides exhibited significant inhibitory activity against BCG at 200 µg/ml [11,32,33]. The isolated triterpenoids namingly: arjunolic acid (1, TAP40), α-amyrin (2, TAPE51) and 2, 3, 23 -trihydroxylolean-12-ene (3, TAP28) were investigated for antimycobacterial activity against BCG as summarized in Table 5. Only compound 1 exhibited moderate activity (MIC 156 µg/mL), when compared to the positive control, INH (MIC 78 µg/mL). From the phytochemical analysis in Table 1, the isolated compounds (1-3) are triterpenoidal derivatives. It could be suggested that compound 1 (arjunolic acid) with triterpenoidal moiety might be important for the observed activity and carboxyl group may be responsible. Previous studies have confirmed that some of the species produces compounds that exert some pharmacological activities like asiatic, oleanolic, betulinic acids, and β-amyrin, which have antimicrobial activities. The related compounds such as lupeol, betulin, and ursolic acid have been found to be antimycobacterial activity. The isolated compound (arjunolic acid) investigated for antimycobacterial activity

against BCG revealed moderate activity against BCG at156 µg/mL. From the results obtained so far, there appears to be a rationale for the use of T. avicennioides to treat bloody sputum and cough in humans. These results provide promising baseline information for the potential use of the isolated compound in the treatment of bacterial infections.

Table 5. Antimycobacterial activity of the three triterpenoids against BCG

Isolated Compound	MIC (µg/mL)
TAP40	156
TAP28	NA
TAPE51	NA
INH	78

MIC→Minimum Inhibitory Concentration, Ta→Terminalia avicennioides extract, NA→No Activity

ACKNOWLEDGEMENTS

AM thanks the Federal Polytechnic, Bida which enabled 3 months' study leave at the SQU, Department of Chemistry. We will also like to thank Drs Hiroko Murata and Yuka Inatomi, both of Setsunsu University, Faculty of Pharmacetical Sciences, Osaka-Japan for the performance of some NMR experiments as well as Mr Mahmud Al-azwani, Department of Chemistry; and Drs Tabisam Khan and Ahlam Al-Azkawi of Central Analytical & Applied Research Faculty (CAARF), all of College of Science, Sultan Qaboos University, Muscat for the NMR and MS spectral analyses respectively. The assistance of the Department of Chemistry, College of Science, Sultan Qaboos University, Muscat, Sultanate of Oman for providing bench space for this work is highly appreciated.

REFERENCES

[1] T.A. Geissman, Flavonoid compounds, tannins, lignins and related compounds. In: M. Florkin and E.H. Stotz (ed.) Pyrrole pigments, isoprenoid compounds, and phenolic plant constituents, vol. 9. Elsevier, New York, New York. p. 265. 1963

[2] Oliver-Bever, B. 1983, Medicinal Plants in tropical West Africa III. Anti-infection therapy with higher plants. J. Ethnopharmcol., 9,1-83

[3] Farnsworth, N.R., and Morris, R.W., 1976, Higher plants–the sleeping giant of drug development. Am. J. Pharm. Edu., 147, 46-52

[4] Tapsell, L.C., Hemphill, I., Cobiac, L., Sullivan, D.R., Clifton, P.M., Williams, P.G., Fazio, V.A., Inge, K.E., Fenech, M., Patch, C.S., Roodenrys, S., and Keogh, J.B., 2006, The health benefit of herbs and spices: the past, the present and the future. Med. J. Aust. Suppl, (Accessed on www.healthyherbs.com)

[5] H.M. Burkill, The useful plants of Tropical Africa, 2nd Ed., Vol.1, Royal Botanic Gardens, Kew, London,1985

[6] A. Mann, M. Gbate, and A. Nda-Umar, Medicinal and Economic Plants of Nupeland, Jube-Evans Books and Publications, Bida, Niger State, Nigeria, 2003

[7] Mann, A., Amupitan, J.O., Oyewale, A.O., Okogun, J.I., Ibrahim, K., 2007, An Ethnobotanical survey of indigenous flora for treating tuberculosis and other respiratory diseases in Niger State, Nigeria. J. Phytomed. & Therap., 12, 1-12

[8] A. Mann, Survey of Ethnomedicine for the treatment of Tuberculosis: Chemistry Perspective. Ayanwola Printing Works, Minna, Niger State, Nigeria, 2007

[9] Abdullahi, A.L., Agho, M.O., Amos, S., Gamaniel, K.S., and Wambebe, C., 2001, Antidiarrhoeal activity of the aqueous extract of Terminalia avicennioides roots. Phytother. Res., 15(5), 431-434

[10] W.H. Lewis, and M.P.F. Elvin-Lewis Medical Botany - plants affecting man's health. John Wiley & Sons, 1977

[11] Mann, A., Ibrahim, K., Oyewale, A.O., Amupitan, J.O., Fatope, M.O., Okogun, J.I., 2011, Antimycobacterial Friedelane-terpenoid from the root bark of Terminalia avicennioides, Am. J. Chem., 1(1), 1-4

[12] Shuaibu, M.N., Wuyep, P.T.A., Yanagi, T., Hirayama, K., Ichinose, A., Tanaka, T., and Kouno, I., 2007, Trypanocidal activity of extracts and compounds from the stem bark of Anogeissus leiocarpus and Terminalia avicennioides. Parasitol. Res., 102 (4), 697-707

[13] Chen, D.F., Zang, S.X., Wang, H.K., 1999, Novel anti-HIV lancilactone C and related triterpenes from Kadsura lancilimba. J. Nat. Prod., 62, 94–97

[14] Paduch, R., Kandefer-Szerszen, M., Trytek, M., and Fiedurek, J., 2007, Terpenes: substances useful in human healthcare. Arch. Immunol. Ther. Exp., 55, 315-327

[15] Kuete, V., Nguemeving, J.R., Beng, V.P., Azebaze, A.G.B., Etoa, F-X., Meyer, M., Bodo B., and Nkengfack, A.E., 2007, Antimicrobial activity of the methanolic extracts and compounds from Vismia laurentii De Wild (Guttiferae). J. Ethnopharm., 109(3), 372-379

[16] Fatope, M.O., Salihu, L., Asante, S.K., and Takeda, Y., 2002, Larvicidal activity of extracts and Triterpenoids from Lantana camara. Pharm. Biol., 40(8), 564-567

[17] K. Hostettmann, and A. Marston, Chemistry and Pharmacology of Natural Products. In: Saponins. Cambridge University Press, Cambridge, U.K. p. 548., 1995

[18] R. Brain, and T. D. Turner, The Practical Evaluation of Phytopharmaceuticals. Wright-Science Technical, Bristol, 1975

[19] J.B. Harborne, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. London, Chapman and Hall Ltd, 1989

[20] G.E. Trease, and W.C. Evans, Pharmacognosy, 13th Ed. London, Bailliere Tindall, 1989

[21] Mahato, S.B., and Kundu, A.P., 1994, 13C NMR spectra of pentacyclic triterpenoids: a compilation and some salient features. Phytochem., 37(6), 1517-1575

[22] Ibezim, E.C., 2005, Microbial resistance to antibiotics. Afr. J. Biotech., 4(13), 1606-1611

[23] Ahmed, I., and Beg, A.Z., 2001, Antimicrobial and

phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J. Ethnopharm., 74, 113-123

[24] C.M. Cotton, Ethnobotany: Principles and Applications. John Wiley & Sons Ltd., Chichester, New York, 1996

[25] Strohl, W.R., 2000, the role of natural products in a modern drug discovery program. Drug Discovery Today, 5(2), 39-41

[26] G. Patrick, Medicinal Chemistry-Instant Notes. (1st Ed) Bios Scientific Publishers Ltd, Leeds, UK, pp. 77-83, 2001

[27] Cowan, M.M. 1999, Plant Products as Antimicrobial Agents. Clin. Microbiol. Rev., 12(4), 564-582

[28] R.E. Schultes, The kingdom of plants. In: W. A. R. Thomson (Ed) Medicines from the Earth. McGraw-Hill Book Co., New York, p. 208, 1978

[29] Copp, B.R., 2003, The Review of Natural Products with antimicrobacterial activity. Nat. Prod. Rep., 20(6), 535-557

[30] Newton, S.M., Lau, C., and Wright, C.W., 2000, A review of natural antimycobacterial products. Phytother. Res., 14, 303-322

[31] Okunade, A.L., Elvin-Lewis, M.P.F., and Lewis, W., 2004, Natural antimycobacterial metabolites: current status. Phytochem., 65, 1017-1032

[32] Mann, A., Amupitan, J.O., Oyewale, A.O., Okogun, J.I., Ibrahim, K., Oladosu, P., Lawson, L., Olajide, I., and Nnamdi, A., 2008, Evaluation of in vitro antimycobacterial activity of Nigerian plants used for treatment of respiratory diseases. Afr. J. Biotech., 7(11), 1630-1636

[33] Mann, A., Ibrahim, K., Oyewale, A.O., Amupitan, J.O., and Okogun, J.I., 2009, Antimycobacterial activity of some medicinal plants in Niger State, Nigeria. Afr. J. Infect. Dis., 3(2), 44-48