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ISOLATION OF LUPEOL, OLEANYL ERUCOATE AND OLEANOLIC-3-ACETATE FROM *CRYPTOLEPIS OBLONGIFOLIA* (MEISN) SCHLTR.

SAIFULLAHI UMAR[1,*], ANAS ABBA[1], MSHELIA EMMANUEL HALILU[2]

1. Department of Pharmacognosy and Herbal Medicine, Bayero University, Kano
2. Department of Pharmacognosy and Ethnobotany, Usmanu Danfodiyo University, Sokoto

ABSTRACT

Cryptolepis oblongifolia (Periplocaceae) is a thin-stem twinning and scrambling shrub with height of 1 m and is locally used in the treatment of malaria, inflammation and stomach disorders. Column chromatography of the n-hexane and ethyl acetate extracts of *C. oblongifolia* led to the isolation of compounds A1, B1 and C1 which were identified using ^{1}HNMR, ^{13}CNMR, DEPT 135, HSQC and HMBC spectra. The melting point of compounds A1, B1 and C1 were found to be 175-176^{o}C, 78-80^{o}C and 216-217^{o} C respectively. Red colouration was observed for the three compounds when tested with Lieberman-Burchard's reagent which indicated the presence of triterpenoid nucleus. The ^{1}HNMR of the compounds (A1, B1 and C1) revealed doublet of doublet around 3.5 ppm. The ^{13}CNMR for A1 indicated unsaturation at 109 and 150 ppm (Lupane group), while B1 indicated saturated oleanane group and C1 indicated unsaturation around 122.0 and 144.0 ppm (Olean-12-ene). The chemistry of Lupeol, Oleanyl erucoate and Oleanolic -3- acetate will help greatly in understanding their pharmacological activities.

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INTRODUCTION

Triterpenoids contain a widely biological group of terpenoids with a large structural diversity of secondary metabolites with more than 100 carbon skeletons identified from terrestrial and marine living organisms [1]. This group of natural products, include triterpenes, steroids limonoids, quassinoids and triterpenoid and steroidal saponins, and is made-up of over 30000 compounds isolated and identified [2]. Most of triterpenic skeletons are tetracycles, containing three six-membered and one five-membered rings, and pentacycles, either with four six membered and one five-membered rings or five six-membered rings. The term triterpene refers to two sesquiterpenes which is up to 30 carbons grouped in six isoprenyl units. It is well-known that triterpenes have long been used as flavors, pigments, polymers, fibres, glues and waxes. In many Asian countries, herbal products containing triterpenes are widely prescribed to prevent or treat a variety of diseases by traditional healers [3]. Lupeyl steryl ether isolated from Nigerian medicinal plant *Paullinia pinnata* has shown to be more active than gentamicin against *Clostridium tetani* with minimal inhibition concentration (MIC) values at 18.4 and 20.7 µg/mL, respectively [4]. Ursolic and oleanolic acids which are pentacyclic triterpenoids were shown to have

***Corresponding author:** sumar.phc@buk.edu.ng; +2347032856542*

significant cytotoxic activity against the A2780 human ovarian cell line [5]. Also, ursolic acid alone has been shown to possess immunomulatory [6], antioxidant [7] and anti-HIV activities [8]. Similarly, Umar *et al*. [9] have reported the inhibitory activity of oleanyl erucoate against Phospholipase A_2 of *Naja nigricollis* venom. The lupane triterpenoids such as betulin, 28-acetoxy betulin, epibetulin, epibetulin acid and betulonic acid have showed anti-inflammatory activity through inhibition of nitric oxide and Prostaglandin E2 production in mouse macrophages stimulated with bacterial endotoxin [10,11].

The aim of the present research was to isolate compounds from *Cryptolepis oblongifolia* that will support its local use in traditional medicine.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The aerial part of *C. oblongifolia* was collected in the month of September, 2019 at Dagachi district of Zaria and identified with a voucher number 302 at Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The samples were shade-dried before size reduction into powder.

Extraction of Plant Material

The powdered plant (1000 g) was extracted via successive maceration with hexane (5.0 L) and ethyl acetate (5.0 L). The extracts were concentrated under reduced pressure with rotary evaporator.

Silica –gel Column Chromatography of Hexane and Ethyl acetate Extracts

The extracts (5 g) each was loaded on to the packed adsorbent and allowed to stabilize for 3-hours before elution. Hexane (100 %) was used as the initial eluent followed by ethyl acetate which was increased gradiently from 0-50 %. Fractions of 40 ml each were collected, allowed to concentrate/dry at room temperature. Column fractions were monitored on TLC plate (Merck F_{254}) visualized under UV 254 nm and 365 nm. P-anisaldehyde reagent and vanillin/sulphuric acid were used as spraying reagent. Total of seventy (70) fractions were collected and merged based on their TLC profiles (colour and number of spots). Further purification was carried out on smaller column chromatography where 200 mg of the fractions X and Y were chromatographed over 20 g of silica gel. Hexane: ethyl acetate (7:3) was used as the eluent at constant composition. This led to the isolation of three compounds (A1, B1 and C1).

Melting Point Determination for Isolated Compounds

The melting point of the isolated compounds was determined using Gallenkamp melting point apparatus and the melting point was ascertained from the thermometer when the sample melted.

Test for Terpenoids (Liebermann - Burchard Test)

The isolated compounds were dissolved in hexane and spotted on TLC plate developed in hexane: ethyl acetate (7:3) solvent system. The plates were sprayed with Lieberman – Burchard's reagent.

Spectroscopic Analysis

The nuclear magnetic resonance (NMR) spectroscopy conducted include 1D (^{1}H, ^{13}C, and DEPT-135) and 2D (H-H COSY, HSQC, and HMBC). The NMR spectra were obtained on ARX-400 MHZ (Brucker/ TOPSPIN) at the University of Pretoria, South Africa.

RESULTS

Column Chromatography

Three compounds were isolated and designated as A1 (100 mg, white colour), B1 (50 mg, white colour) from hexane extract and C1 (50 mg, white colour) from ethyl acetate extract.

Determination of Melting Point

The melting point of compounds A1, B1 and C1 was found to be 175-176^o C, 78-80^o C and 216-217^o C respectively.

Liebermann-Burchard Test

The test showed a red colouration for all the compounds (A1, B1 and C1) indicating the presence of triterpenoid.

DISCUSSION

The column chromatography of the extracts afforded two compounds from hexane (A1 and B1) and one from ethyl acetate (C1). Compound A1 appeared as white amorphous substance which shows red colour with Lieberman Burchard's reagent and melts at 175^{o}C -176^{o}C. The ^{1}HNMR spectrum of compound A1 showed signals due to six methyl singlets at δ_H 0.78 (3H), 0.81(3H), 0.86 (3H), 0.99 (3H), 1.05 (3H) and 1.28 (3H) integrated for 3H each, an olefinic methyl group at δ_H 1.67 (3H), and a doublet due to a terminal methylene protons at δ_H 4.66, H-29b and 4.54 ppm H-29a, which are typical of triterpenoids. The ^{1}HNMR spectrum also showed double doublet at

δ_H 3.20, H-3 due to a methine proton attached to hydroxyl group. The structure assignment of A1 was further substantiated by the ^{13}C NMR experiment which showed seven methyl group [δ_C: 28.0 (C-23), 18.2 (C-28), 16.2 (C-25), 16.2 (C-26), 15.5 (C-25), 14.9 (C-27) and 19.5 (C-30)]; the presence of exomethylene was observed at [δ_C 151.17 (C-20) and δ_C 109.5(C-29)] and methine hydroxy bearing carbon atom at δ_C 79.2 ppm, C-3. Ten methylene and five quaternary carbons were assigned with the aid of DEPT 135. The structure was further established with the aid of 2D NMR experiment (^1H-^1H COSY and HMBC). The COSY spectrum of A1 exhibited some cross peaks such as between (δ_H 2.14, H-19) and one sp^3 methylene proton signal (δ_H 1.37, H-21) and between oxygenated methine proton (δ_H 3.2, H-3) and sp3 methylene signal (δ_H 1.61, H-23). The HMBC spectrum showed cross peaks between methine proton signal at δ_H 3.2 (H-3) and methyl carbon signal (δ_C 28.0 C-23), the pair of broad singlets of olefinic proton at δ_H 4.55 and 4.66 showed cross peak with methylene carbon signal [δ_C 48.2 (C-19) and 19.5 (C-30)]. By comparison with reported data literature as showed in Table 1, compound A1 was proposed to be lupeol, a pentacyclic tri-terpenoid (Figure 1).

Table 1: ^{1}HNMR and ^{13}CNMR Spectral Data of Compound A1 and Literature reported in ppm (in CDCl3)

C/H S/NO	^{13}CNMR	^{1}HNMR	^{13*}CNMR	^{1*}HNMR
1.	38.8	-	38.7	
2.	27.4	-	27.4	
3.	79.2	3.21	79.0	3.21
4.	38.9	-	38.9	
5.	55.5	0.71	55.5	0.71
6.	18.5	1.39	18.5	1.39
7.	34.4		34.2	
8.	41.0		40.9	
9	50.6	1.28	50.5	1.28
10.	37.3		37.2	
11.	21.1	1.53	21.0	1.53
12.	25.3	1.28	25.2	1.29
13.	38.3	1.67	38.1	1.67
14.	42.5	1.42	42.9	1.42
15.	27.2	1.05	27.1	1.05
16.	35.8		35.5	
17.	43.0		43.0	
18.	48.5	0.91	48.3	0.91
19.	48.2	2.41	48.0	2.14
20.	151.1		151.0	
21.	30.0	1.33	29.9	1.33
22.	40.0		40.2	
23.	28.0	1.63	28.2	1.64
24.	15.5	1.61	15.5	1.61
25.	16.2	0.83	16.1	0.84
26.	16.2		16.0	
27.	14.9	0.97	14.8	0.97
28.	18.2	0.78	18.0	0.79
29a.	109.5	4.55	109.0	4.61
29b.	109.5	4. 66	109.0	4.71
30.	19.5	1.67	19.5	1.69

Note: ^{13*}CNMR and ^{1*}HNMR = Abdullahi *et al.* , [11]

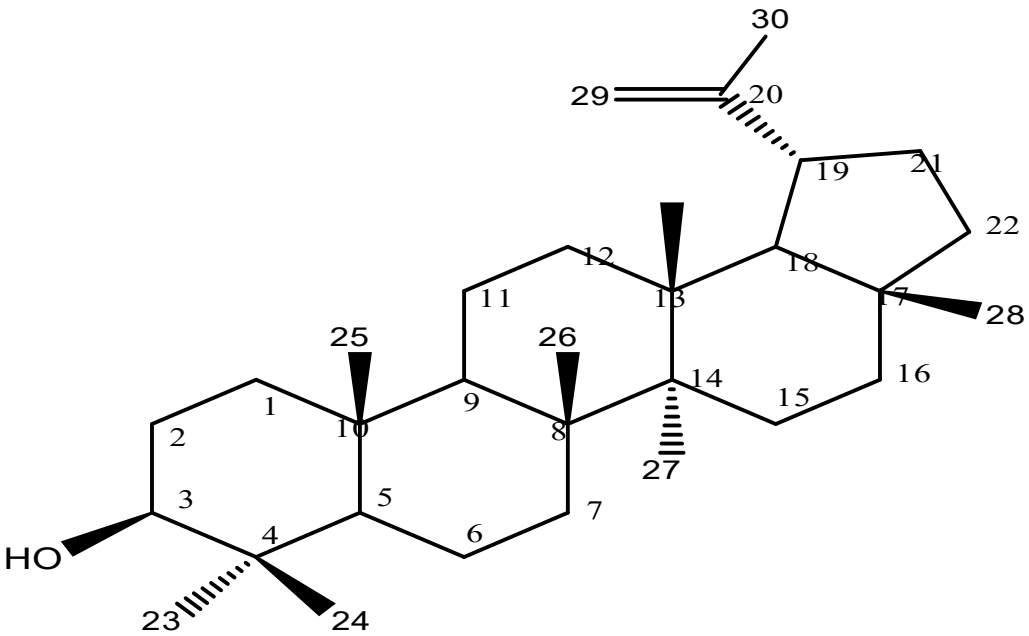

Figure 1: Proposed Structure of Compound A1 (Lupeol)

Compound B1 was obtained as white amorphous substance m.p 79- 80 ^{0}C which gave positive Liebermann- Burchard test for terpenoids, the ^{13}CNMR spectra at 173.8 (C-1') ppm indicating the presence of carbonyl ester. Its ^{1}HNMR spectrum shows the presence nine methyl singlets at δ_H 1.08 (H-23), 0.95 (H-24), 0.96 (H-25), 0.97 (H-26), 0.90 (H-27), 0.99 (H-28), 0.95 (H-29), 1.07 (H-30) and 2.2 (H-21') ppm and a doublet of doublet at δ 4.49 ppm suggesting the basic skeleton of 3β substituted derivative of triterpenoids, also a multiplet at δ_H 2.28 (H-2') indicating a side chain attached to carbonyl carbon atom. The below ^{13}CNMR data was almost the same as that of oleanane. Similarly, the HSQC spectrum shows correlation between methylene protons adjacent to carbonyl of an ester suggesting the presence of a side chain at C-3. The HMBC spectrum showed long range correlation between proton at δ_H 2.28 (H-2') and δ_C 173.7 (C-1') which is a carbonyl; this confirm that it is part of an ester side chain. A broad singlet at δ_H 1.66 ppm (H-1) for β-methylene proton correlates with carbon at δ_C 80.8 ppm (C-3). Based on the above spectra data, HSQC and HMBC correlation and comparison with reported literature (Table 2), compound B1 was proposed to be oleanyl erucoate (Figure 2).

Table 2: ^{1}HNMR and ^{13}CNMR Spectral Data of Compound B1 and Literature reported in ppm (in CDCl3)

C/H S/NO	^{13}CNMR	^{1}HNMR	^{13}*CNMR	^{1}*HNMR
1.	38.6	1.05, 1.66 m	38.7	1.05, 1.68 m
2.	27.6	1.23 m	27.4	1.26 m
3.	80.8	4.46	78.8	4.50
4.	38.8		38.8	
5.	55.5	0.82 m	55.2	0.82 m
6.	18.3	1.34 m	18.3	1.30 m
7.	34.5	1.51 m	34.4	1.52 m
8.	40.2		40.8	
9.	51.3	1.58 m	50.1	1.57 m
10.	37.3		37.1	
11.	21.3	1.70 m	20.9	1.87 m
12.	26.4	1.76 m	26.8	1.90 m
13.	37.9		37.8	
14.	43.0		43.1	

Table 2 continued

C/H S/NO	^{13}CNMR	^{1}HNMR	^{13}*CNMR	^{1}*HNMR
15.	27.4	1.34 m	27.4	1.34 m
16.	35.0	1.25 m	35.5	1.30 m
17.	43.5		43.1	
18.	18.3	1.38 m	18.3	1.35 m
19.	43.5	0.98 m	44.6	0.98 m
20.	29.4		29.3	
21.	21.3		21.9	
22.	40.2	1.34 m	40.4	1.35 m
23.	28.2		28.0	
24.	15.7	0.95 s	15.4	0.93 s
25.	16.2		16.0	
26.	16.3		16.0	
27.	14.7	0.90 s	14.4	0.90 s
28.	18.2		18.0	
29.	15.7		15.1	
30.	23.7		23.0	
1'.	173.8		173.7	
2'.	34.9	2.28 t	34.9	2.29 t
3'.	25.4	1.74 m	25.2	1.74 m
4-11'	29.4-29.9	1.23 brs	29.2-29.7	1.28 brs
12'	32.1		29.7	
13'	32.3		29.7	
14'	32.4		29.7	
15'	37.6		29.7	
16'	38.3		22.7	
17'	15.5	0.91 m	14.1	0.89 t
18'	51.3	1.42 m	50.9	
19'	55.5	1.76 m	54.6	
20'	142.9		141.9	
21'	76.9	2.2 m	77.0	
22'	130	4.84 s	129.2	

Note: ^{13}*CNMR and ^{1}*HNMR = [9]

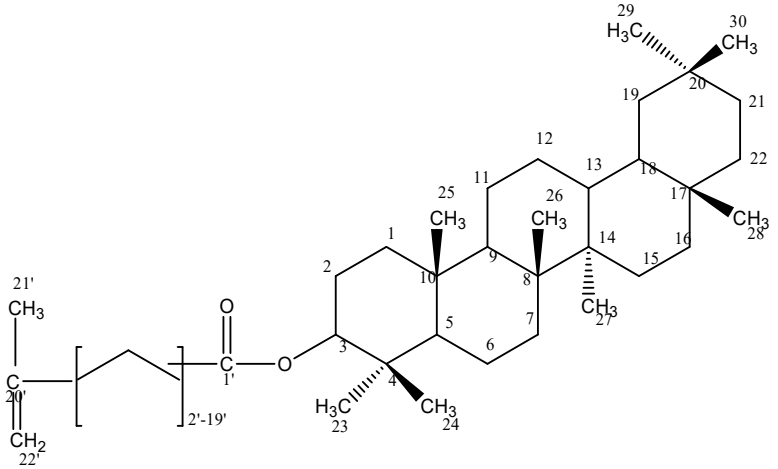

Figure 2: Proposed Structure of Compound B1 (Oleanyl Erucoate)

Compound C1 was obtained as white crystal with melting point 217°C-218°C and showed positive with Liebermann Burchard test. The ^{13}CNMR spectral data at δc 184.2 ppm revealed the presence of carboxylic acid, also at δc 171.5 ppm indicating the presence of carbonyl esters (C-31). The ^{1}HNMR spectrum shows the presence of eight quaternary methyl at δ_H 1.01 (H-23), 0.80 (H-24), 0.98 (H-25), 1.16 (H-26) 1.16 (H-27), 0.91 (H-29), 0.97 (H-30) and 2.14 (H-31), ppm. Also, a doublet at δ_H 4.49 ppm suggests the basic skeleton of 3β substituted pentacyclic triterpene derivative. Also, a doublet was observed at δ_H 4.49 ppm for hydrogen bearing methyl acetate (C-3). The ^{13}CNMR spectral data suggested a triterpenoid (oleanolic acid). The HSQC spectrum shows correlation between methine carbon atom (δc 122.3/C-12) and methine proton (δ_H 5.25/H-12) and also methine bearing ester group (δc 81.1/C-3) and methine proton (δ_H4.49/H-3). Also, the HMBC spectrum showed long range correlation between C-3 (δc 81.1) and H-24 (δ_H 0.80), C-5 (δc 55.5) and H-25 (δ_H 0.72). Based on these spectral data and what are obtainable from literature as showed in Table 3, compound C1 was proposed to be oleanolic -3-acetate (Figure 3).

Table 3: ^{1}HNMR Spectral Data of Compound C1 and Reported Literature (in $CDCl_3$)

C/H S/NO	^{13}CNMR	^{1}HNMR	^{13*}CNMR	^{1*}HNMR
1.	38.3	1.63	38.4	1.62
2.	23.6	1.60	23.6	1.60
3.	81.1	-	81.1	-
4.	37.9	-	37.7	-
5.	55.5	0.72	55.2	0.74
6.	18.5	1.54	18.1	1.54
7.	32.7	1.49	32.4	1.49
8.	39.2	-	39.5	-
9	47.5	1.55	47.7	1.54
10.	36.9	-	37.2	-
11.	23.6	0.91	23.5	0.94
12.	122.7	5.25	122.7	5.31
13.	143.8	-	143.6	-
14.	41.7	-	41.5	-
15.	26.1	1.60	25.9	1.60
16.	27.6	0.97	27.8	0.94
17.	32.4	-	32.6	-
18.	46.5	2.87	46.7	2.82
19.	41.1	2.88	40.8	2.87
20.	45.8	-	46.0	-
21.	29.9	1.62	29.7	1.62
22.	37.2	1.31	36.9	1.30
23.	28.0	1.01	28.2	1.00
24.	16.8	0.80	16.7	0.78
25.	15.3	0.98	15.5	0.93
26.	17.1	1.16	17.3	1.12
27.	28.0	1.16	28.2	1.16
28.	184.2	1.02	184.3	1.00
29.	33.0	0.91	33.2	0.92
30.	19.5	0.97	18.4	0.94
31.	171.5		171.23	

Note: ^{13*}CNMR and ^{1*}HNMR = [12]

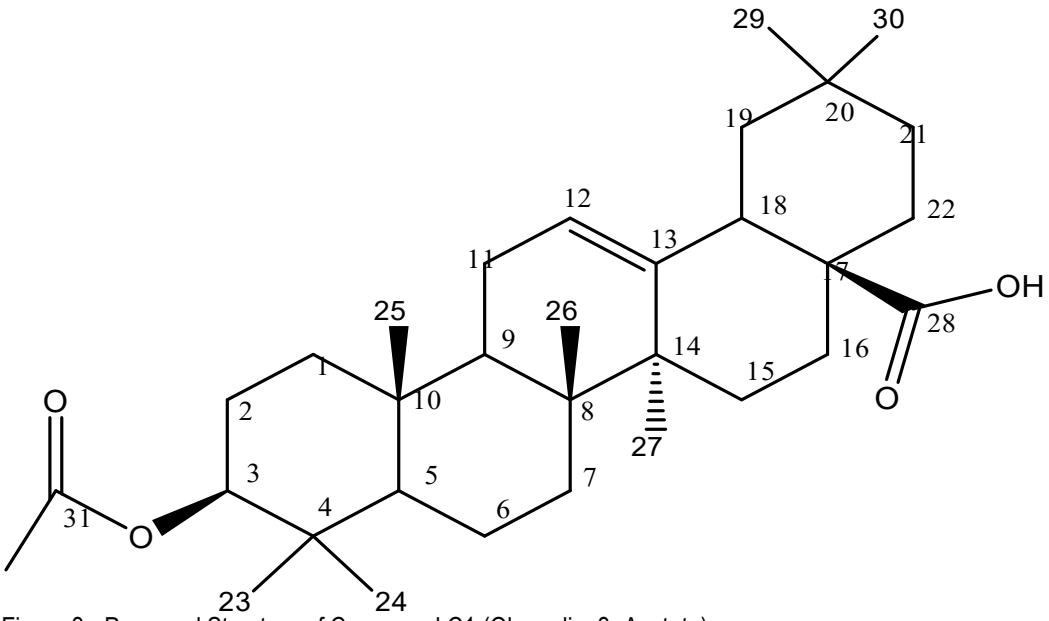

Figure 3:- Proposed Structure of Compound C1 (Oleanolic -3- Acetate)

CONCLUSION

Several triterpenoids were known with different kind of skeletons such as sterol pentacyclic, tetracyclic, glycosides and polyhydroxylated triterpenoids isolated from Nigerian medicinal plants. Some of these compounds have proven to have well-defined pharmacological activities. Some activities are moderate while others have significant activities as compared with the standard drug. Compounds like lupeol, oleanyl erucoate and oleanolic-3-acetate can serve as potential drug or as raw materials for semisynthetic compounds that are of therapeutic importance in orthodox medicine.

REFERENCES

1. Mahato SB, Nandy AK, Roy G. Triterpenoids. Phytochemistry 31(7), 1992: 2199-249.
2. Dzubake P, Hajduch M, Vydra D, hustov A, Kvasnica M, Biedermann D. Pharmacological activities of natural triterpenoids and their therapeutic implications Natural. product rep 23(3), 2006: 394-411.
3. Xu R, Fazio GC, Matsuda SPT. On the origins of triterpenoid skeletal diversity. Phytochemistry, 65, (2004). 261-291.
4. Lasisi AA, Ayinde BW, Adeleye AO, Onocha PA, Oladosu IA, Idowu PA. New triterpene isovanniloyl and antibacterial activity of constituents from the roots of *Paullinia pinnata* Linn (Sapindaceae). Journal of Saudi Chemical Society. 10, 2012:1016-102.
5. Murphy BT, Brodie P, Slenbodnick C, Miller JS, Birkinshaw C, Randrianjanaka LM. Antiproliferative limonoids of a *Malleastrum* Sp from the Madagascar rainforest. *Journal of Natural products* 71(3), 2008: 325-9.
6. Jang SM, Yee ST, Choi J, Choi MS, Do GM, Jeon SM, Yeo J, Kim MJ, Seo KI, Lee MK. Ursolic acid enhances the cellular immune system and pancreatic β-cell function in strepto-zotocin-induced diabetic mice fed a high fat diet. International Immunopharmacology 9, 2009: 113-119.
7. Ramachandran S, Prasad NR. Effect of ursolic acid, a triterpenoid antioxidant, on ultraviolet- radiation-induced cytotoxicity, lipid peroxidation and DNA damage in human lympho-cytes. Chemico-Biological Interactions 176, 2008: 99-107.
8. Lee JS, Miyashiro H, Nakamura N, Hattori M. Two new triterpenes from the rhizome of *Dryopteris crassirhizoma*, and inhibitory activities of its constituents on human immunod-eficiency virus-1 protease. Chemical and Pharmaceutical Bulletin 56, 2008a: 711-714.
9. Umar S. A. Ahmed, H. Ibrahim, AB Sallau, and October Natasha. Inhibitor from *Cryptolepis oblongifolia*. Journal of Natural Sciences Research 4 (4), 2014: 63-67.

10. Misko TP, Schilling RJ, Salvemini Salvemini D, Moore WM, Currie MG. Activity of lupane triter-penoids from *Maytenus* species. Analytical Biochemistry 214, 1993: 11.
11. Moroney MA, Alcaraz MJ, Forder RA, Carey F, Hoult JRS. Activity of lupane triterpenoids from Maytenus species. Journal of Pharmacy and Pharmacology 40, 1988: 787.
12. Abdullahi, S.M., Musa, A.M., Abdullahi, M.I., Sule, M.I and Sani Y.M., Isolation of lupeol from the stem-bark of *Lonchocarpus sericeus* (Papilionaceus). Scholars Acedemic Journal of Biosciences (SAJB) 1(1), 2013:18-19.