ISOLATION AND CHARACTERIZATION OF LUP-20(29)-EN-3B-OL (LUPEOL) FROM THE LEAF OF *Combretum lamprocarpum* DIELS (COMBRETACEAE)

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Abstract: The purpose of this work was to isolate and characterize the bioactive compound from the leaf of *Combretum lamprocarpum* Diels (*Combretaceae*). The powdered plant material was extracted using Microwave-Assisted Extraction technique and n-hexane, chloroform, ethyl acetate, and methanol as solvents. The ethyl acetate extract was loaded on a silica gel column using n-hexane and ethyl acetate as eluents. A pure compound was isolated and its Infra-Red, proton and carbon 13 Nuclear Magnetic Resonance spectra were measured. A pair of broad singlet protons appeared in the proton Nuclear Magnetic Resonance spectra at δ 4.54 and 4.67 (1H each). This was indicative characterization of the exo-methylene group present in the Lupane type of triterpenoids. The compound was characterized as Lupeol by comparing its carbon 13 Nuclear Magnetic Resonance spectroscopic data to the literature data. A compound, Lupeol (Lup-20(29)-en-3β-ol) was isolated from the ethyl acetate extract of the leaf of *Combretum lamprocarpum*. Thorough literature search shows that this compound was isolated from this plant for the first time.

Keywords: *Combretum lamprocarpum*, leaf, triterpenoid, lupeol, ethyl acetate, Combretaceae

Introduction

The plant *Combretum lamprocarpum* Diels belongs to the family, Combretaceae, made up of approximately 600 species. They are widely distributed in the tropical and sub-tropical regions of Africa (Roy *et al.,* 2014). Some species from this genus have been used as ethno-medicinal agents in the treatment of wounds, stomach ache (Gibreel, 2013), vomiting (Adjakpa *et al.,* 2016), diarrhoea (Offiah *et al.,* 2012), inflammation, tumour and cancer (Petronella *et al.,* 2009). Preliminary phytochemical studies of combretum species have shown the occurrence of alkaloids, terpenoids, tannins and saponins (Mbwambo *et al.,* 2013), saponins, alkaloids, phenolic, tannins, glycosides, flavonoids and anthraquinones (Yahaya *et al.,* 2012).

A number of bioactive compounds have been isolated from the genus Combretum, and most of these isolated compounds belong to the class of terpenoids, steroids, flavonoids, and stilbenes (Eloff *et al.,* 2008). The isolation of cardamonin was reported by Aderogba *et al.* (2012) from *Combretum apiculatum.* An important polyphenol, 3-*O*-methyl quercetin (Araujo *et al.,* 2013) was isolated from the methanol extract of the flowers of *Combretum lanceolatum*. Also combretastatins (Fyhrquist *et al.,* 2006), have been isolated from several species of Combretum. Betulinic acid has been isolated from *C. quadrangulare, C. laxum* and *C. yunnanense* (Bisoli *et al.,* 2008; Wang *et al.,* 2011). Also, a flavan, 3′,4′,5,7-tetrahydroxyflavan was isolated from *Combretum erythrophyllum* (Schwikkard *et al.,* 2000). A triterpene, arjunolic acid which showed *in vitro* activity against snake venom has been isolated from the ethanolic root extract of *Combretum leprosum* (Fernandes *et al.,* 2014).

Materials and Methods

Collection of plant materials

The leaf of *Combretum lamprocarpum* Diels was collected from Makurdi, Benue State, Nigeria. It was authenticated by Mr. Namadi Sanusi of the Herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, where a specimen voucher number (900743) was deposited. The leaf was air-dried and ground into powder using a wooden mortar and pestle, sealed in polyethene bag and kept in a desiccator before the extraction.

Microwave extraction procedure

The pulverized plant material (1 kg) was divided into three different portions (333.33 g each) and placed in mason jars. N-hexane was added to the three portions until it just covered the top. The bottles were covered tightly and then irradiated in the microwave oven (Kenwood K25MSS11) under 150 N for 3 min. The bottles were removed from the oven and allowed to cool and vented. The process was repeated 5 times. The samples were washed 3 times with N-hexane and filtered through muslin cloth. The procedure was repeated using chloroform, ethyl acetate, and methanol, respectively. Each of the extracts was concentrated in a rotary evaporator at 40°C.

Isolation of compound

General instrumentation

The microwave-assisted extraction (MAE) of the pulverized plant material was carried in (Kenwood K25MSS11) commercial microwave oven. The extracts were recovered using rotary evaporator (BUCHI RE110). The spots on the TLC (silica gel 60. F254 Merk 0.2 mm) plates were visualized under UV lamp 254-326 nm (Hitachi U-3200). Gallenkamp oven (OV-440) was used to dry the developed TLC plates. The ^{1}H and ^{13}C analysis was carried out on Agilent-NMR-vnmrs400 spectrometer. Deuterated chloroform ($CDCl_3$) relative to TMS as internal reference. The FTIR Agilent Technologies Cary 630 FTIR spectrometer was used to run the IR. The melting was measured using digital melting point apparatus (Stuart SMP40).

Results and Discussion

A pure white crystalline solid compound was isolated and labelled, K1 (Fig. 3). It had a melting point of 213-215°C. The ^{13}C-NMR spectrum show a total of 30 carbon atoms, a characteristics of triterpenoid structure.

The proton NMR spectrum of compound K1 has revealed the presence of seven methyl at δ 0.77 (3H, d), 0.84(3H, s), 0.96(3H, d), 1.047(3H, s), 1.28 (3H, s), 1.40 (3H, d) and 1.66(3H, s) in (ppm). And a pair of singlets at δ4.54 and 4.67 (1H each). The presence of secondary hydroxyl group at δ 3.18 (dd, J=11.2, 4.8 Hz).

The results obtained for IR, ^{1}H-NMR and ^{13}C-NMR are represented as follows:

Spectral data

The IR spectrum of K1 showed bands at 3403, 2926, 2855 cm^{-1} and other bands in the finger print region. The ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.77 (3H, d), 0.84(3H, s), 0.96(3H, d),

1.047(3H, s), 1.28 (3H, s), 1.40 (3H, d), 1.66(3H, s), δ4.54 and 4.67 (1H each)/C-29, δ 3.18 (dd, J=11.2, 4.8 Hz)/ C-3.
The ^{13}C-NMR ($CDCl_3$, 400 MHz) δ 150.98 (C- 20),109.32 (C-29), 78.89 (C-3), 17.98 (C-28), 55.22 (C-5), 50.37 (C-9), 48.23 (C-18), 47.65 (C-17), 47.96 (C-19), 42.98 (C-14), 41.49 (C-8), 39.97 (C-1), 38.64 (C-4), 37.12 (C-10), 37.98 (C-13), 34.21 (C-7), 31.92 (C-22), 29.70 (C-21), 35.54 (C-16), 28.07 (C-23), 27.96 (C-2), 27.40 (C- 15), 25.06 (C-12), 20.88 (C-11), 19.28 (C-30), 18.28 (C-6), 16.11 (C-25), 16.93 (C-26), 15.36 (C- 24), 14.52 (C-27).

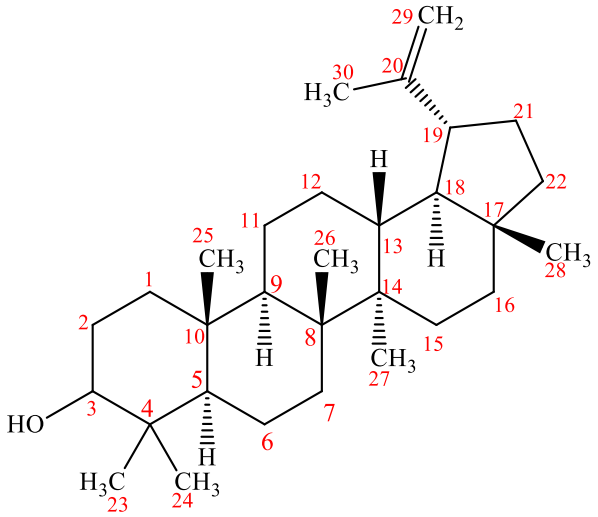

Fig. 1: Chemical structure of Lupeol

The compound was obtained as a white powdery compound with a melting point of 213-215ºC. The IR spectrum of compound K1 showed absorption bands characteristic of the hydroxyl group at (3403 cm^{-1}). The presence of terminal double bond (representing the exocyclic double bond) was confirmed by bands at 2926 and 2855 cm^{-1}.
The ^{1}H-NMR (Fig. 2) spectrum of compound K1 showed the presence of seven methyl groups at δ 0.77 (3H, d), 0.84(3H, s), 0.96(3H, d), 1.047(3H, s), 1.28 (3H, s), 1.40 (3H, d) and 1.66(3H, s) all in (ppm). Also, a pair of singlets at δ4.54 and 4.67 (1H each) was due to the vinyl protons at carbon 29. The presence of a doublet of a doublet with one proton intensity at δ 3.18 (dd, J=11.2, 4.8 Hz) was seen which was due to the proton attached to the carbon bearing the hydroxyl group at C-3. These characteristics indicated that compound K1 belongs to the lupane class of triterpenoids (Abdullahi *et al.*, 2013).

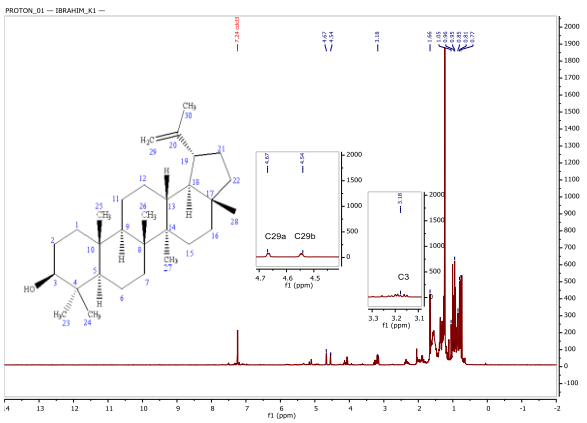

Fig. 2: ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$) of compound K1

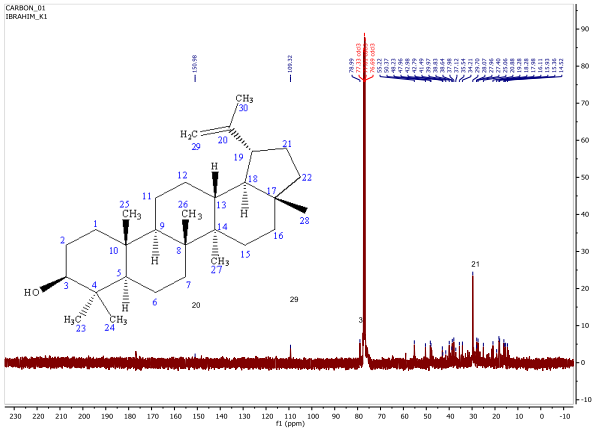

Fig. 3: ^{13}C NMR spectrum of compound K1

The ^{13}C NMR (Fig. 3) of the compound K1 showed 30 signals. The observed signals were; seven methyl groups [δ 28.07 (C-23), 17.98 (C-28), 16.11 (C- 25), 16.93 (C-26), 15.36 (C-24), 14.52 (C-27) 19.28 (C-30)]. Also, ten methylenes, five methines and five quaternary carbons. The characteristic sp^2 carbon signals of lupeol are observed at δ 150.98 and 109.32 ppm (C-20 and C-29), respectively and a hydroxyl group bonded to (C-3) at 78.99 ppm. All together accounts for the 30 carbon signals. These spectroscopic data (Table 1) are in line with the literature (Sánchez-Burgos *et al.*, 2015). The comprehensive literature survey and comparison of different spectral data showed by compound K1 led us to elucidate the compound as lupeol (Fig. 1).

Table 1: Comparison of 1D NMR data of K1 with literature data in $CDCl_3$

Carbon Position	^{13}C δ(ppm) (Isolated K1)	^{13}C δ(ppm) (Sánchez-burgos *et al.*, 2015).	^{13}C δ(ppm) (Roy *et al.*, 2016)	Carbon type
C-1	38.83	38.76	38.2	CH_2
C-2	27.96	27.42	25.3	CH_2
C-3	78.89	78.98	79.2	CH
C-4	38.64	38.84	38.9	C
C-5	55.22	55.28	55.5	CH
C-6	18.28	18.30	18.5	CH_2
C-7	34.21	34.23	34.5	CH_2
C-8	41.49	40.80	41.0	C
C-9	50.37	50.40	50.6	CH
C-10	37.12	37.14	37.3	C
C-11	20.88	20.90	21.1	CH_2
C-12	25.06	25.10	27.5	CH_2
C-13	37.98	38.02	39.0	CH
C-14	42.98	42.80	43.0	C
C-15	27.40	27.39	27.6	CH_2
C-16	35.54	35.56	35.8	CH_2
C-17	42.79	42.98	43.2	C
C-18	48.23	48.27	48.5	CH
C-19	47.96	47.96	48.1	CH
C-20	150.98	150.96	151.1	C
C-21	29.70	29.82	30.0	CH_2
C-22	39.97	39.98	40.2	CH_2
C-23	28.07	28.07	28.2	CH_3
C-24	15.36	15.36	15.6	CH_3
C-25	16.11	16.10	16.3	CH_3
C-26	16.93	15.95	16.2	CH_3
C-27	14.52	14.53	14.7	CH_3
C-28	17.98	17.98	18.2	CH_3
C-29	109.32	109.32	109.5	CH_2
C-30	19.28	19.28	19.5	CH_3

Conclusion

The phytochemical investigation of the ethyl acetate extract of the leaf of *Combretum lamprocarpum* Diels was carried out successfully. A known pharmacologically active triterpenoid, Lupeol have been isolated and characterized as lup-20(29)-ene-3β-ol. This compound has been reported to have, anti-inflammatory (Gallo and Sarachine, 2009), anticancer (Petronella *et al.*, 2009; Saleem, 2009), activities.

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Conflict of interest

The authors declare no conflict of interest.

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