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Isolation of Lupeol from the Stem Bark of *Leptadenia hastata* (Pers.) Decne

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ABSTRACT: Dried stem bark powder of *Leptadania hastata* was subjected to maceration with methanol to afford crude methanol extract, which was partitioned with n-hexane, ethylacetate, chloroform and n- butanol to afford different their respective fractions. Extensive phytochemical screening of the n-hexane fraction using column chromatography resulted to the isolation of a white solid substance. The substance was identified as of lupeol using IR, 1D – NMR, 2D – NMR data and by comparison with reference spectral data.

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Leptadenia hastata (Pers.) Decne. (*Asclepiadaceae*) is an important emergent local food of Africa with the ability to grow under harsh environmental conditions, is a widely distributed tropical African herb used as vegetable (Sena *et al*., 1998). *L. hastata* is edible non-domesticated vegetable and it is collected in wild throughout Africa. It is typically grown in tropical dry lands in sandy soil. Wild foods like *L. hastata* provide food security during seasonal changes and are used medicinally in many areas as anti-snake venoms, analgesics, anti-inflammatory, anti tumors, anti-hypertensive and anti-diabetic among others (Thomas, 2012). *Leptadenia hastata* (Pers.) Decne (*L. hastata*) is a perennial species of the *Apocynaceae* family that includes the subfamily *Asclepediaceae*, (Meve and Liede, 2004). *Asclepiediaceae* plants are widely used in traditional medicine and have been reported to be rich in steriodial glycosides, cardenolides, flavonoids, triterpenes and polyoxypregnanederivatives (Bazzaz and Haririzadeh, 2003; Atta and Mouneir, 2005; Cioffi *et al*., 2006). The family is mostly herbs and shrubs with white sap comprising about 250 genera and 2,000 species, many of which are lianous and some of which are cactus like succulents with reduced leaves (Thomas, 2012). Six new polyoxypregnane esters and three new glycosides together with five known esters were separated, purified and elucidated from chloroform extract of the bark of *L. hastata* (Aquino *et al*., 1996). Triterpene has been isolated from the latex of the leaves of *L. hastata* (Nikeima *et al*., 2001). β – sitosterol has been isolated from the methanol leaves extract of *Leptadenia hastata* and stigmasterol glycoside has been isolated from the methanol root bark extract of *Leptadenia hastata* (Mailafiya *et al*., 2017; Mailafiya *et al.,* 2020). Seven flavonoids were isolated from the butanol fraction of the methanol extract of the aerial parts of *Cynanchum acutum L.* which belongs to the same family with *Leptadenia hastata* (*Asclepiadaceae*). All of which have been isolated for the first time from the genus *Cynanchum*. Their structures were established as quercetin 3-*O*-galacturonopyranoside , quercetin 7-*O*- *α* -glucopyranoside , tamarixtin 3-*O*-galacturonopyranoside, kaempferol 3-*O*-galacturonopyranoside , 8-hydroxyquercetin 3-*O* -galactopyranoside , tamarixtin 3-*O*-*α*-rhamnopyranoside, and tamarixtin 7-*O*-*α*-arabinopyranoside on the basis of their chromatographic properties, chemical and spectroscopic data (Ghada *et al.,* 2008). A pentacyclic triterpenoid compound has been reported to be isolated from the fruits of *Dregea volubilis* Benth *Asclepiadaceae (*Bikash and Haldar, 2009). Lupeol, a phytosterol and triterpene, is widely found in edible fruits and vegetables and there is a growing interest in natural triterpenoids due to their wide spectrum of biological activities. Various in vitro and pre clinical animal studies suggests that lupeol has a potential to act as an anti-inflammatory, anti-cancer, anti-microbial, anti protozoal, anti-proliferative, anti-

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invasive, anti-angiogenic and cholesterol lowering agent (Siddique and Saleem, 2011).

The literature survey reveals that there is no report on isolation and elucidation of any pentacyclic triterpene from the stem bark of *L. hastata*. However, the present work reports herein the isolation and identification of lupeol for the first time from hexane fraction of the methanol stem bark of *L. hastata.*

MATERIALS AND METHODS

Collection and Identification of Plant Material: The plant sample of *Leptadenia hastata* was collected in July 2015 at Zaria Local Government area of Kaduna State of Nigeria. It was identified at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria Nigeria by comparing with herbarium reference voucher specimen (No. 900220). The stem bark was shade dried, pounded to powder, labelled and stored for use.

*Preparation of extract:*The powdered stem bark (4.3 kg) was extracted with 90 % methanol using maceration method. The extract was concentrated*in-vacuo* using rotary evaporator at 40^0C to yeild athe crude stem methanol extract (118 g). A portion methanol extract (92 g) was suspended in distilled water and partitioned exhasutively and successively with n-hexane, chloroform, ethylacetate and n-butanolto obtain hexane fraction, chlorofom fraction, ethylacetate fraction and n-butanol fraction respectively. The hexane fraction was subjected to colum chromatography.

*Isolation by column chromatography:*The hexane fraction (5 g) was weighed and subjected to extensive column chromatography; 5 ml of methanol was added to dissolve the fraction. It was followed by addition of silica gel, dried and crushed into fine powder with mortar and pestle before it was mounted on the column.The column was packed with n-Hexane and silica gel using wet slurry method. The sample was mounted on top of the silica gel, but prevented from having direct contact with the silica gel by the use of cotton. Wet method of packing was used in packing the column. The column was eluted using hexane, hexane/ethylacetate as gradient mixture solvent systems of increasing polarity. Several eluates (30 mls) collected were monitored using TLC, and those with similar TLC profile were pooled together as a fractions, evaporated at reduced pressure coded A – K.Further purification of fraction D (295 mg) was carried out by subjection to extensive column chromatography. Exactly 2 ml of methanol was added to dissolve fraction 'D', followed by addition of silica gel, dried and crushed into fine powder with mortar and pestle before it was mounted to the column. The column was packed using n-hexane and silica gel using wet slurry method, the sample was mounted directly on top of the silica gel but prevented from having direct contact with the eluting solvent by use of cotton.The column was eluted using 100 % n- hexane; hexane/ethylacetate. Several eluates (5 ml) were collected and monitored using TLC. Those with similar TLC profile were pooled together as sub-fractions, evaporated at reduced pressure coded D1 – D7.

Purification of isolated compound by gel filtration column chromatography: D4 - D6 were subjected to gel filtration using sephadex LH-20 and methanol as eluting solvent. Two (2) ml each of a total of 6 collections were made and combined based on their TLC profile which led to the isolation of Compound M_2. $M_2$was subjected to ^{1}H-NMR, ^{13}C-NMR and 2D-NMR to ascertain the chemical structure.

RESULTS AND DISCUSSION

Solubility Profile, Chemical Tests and Melting point of M_2: The compound gave a violet single spot on the TLC plate with Rf value of 0.36, it was soluble in methanol, and a positive Liebermann Buchard test. M_2 is a white amorphous solid substance with a melting point of 214^0C. *Spectral Analysis:* IR Vmax cm^{-1} of M_2:The IR spectrum of M_2showed broad absorption bands at 3433cm-1 for hydrogen bonded OH stretch, for C-H stretch in CH_2 and CH_3 at 2942cm-1 and 2357 cm -1 respectively, 1663 cm -1 for C = C symmetric stretch, 1564 cm -1 for C=C asymmetric stretch, 1417 cm -1 for C – H deformation in CH_2 and CH_3, 1035 cm-1 (C – O stretch of Secondary alcohol), = C – H bonding exocyclic CH_2at 889 cm-1. ^{1}HNMR (500 MHz, Deuterated chloroform) of M_2: The proton spectrum of M_2 showed signals at δ 4.69ppm, 4.58 (H – 29, d, d, 2H), 2.43 (H-19, m, 1H),1.43 (H-18, t, H), 1.10 (H-15, d. 1H), 3.16 (H-3, t, 1H), 0.93 (H-23, s. 3H), 0.75 (H-5, t, 1H), 1.68 (H-30, s, 3H) (Table 1). ^{13}C-NMR of M_2: The ^{13}C-NMR spectrum of M_2 revealed the presence of 30 carbon signals at 38.43, 26.64, 79.82, 40.22, 152.10, 110.28 e.t.c (Table 1). APT of M_2: The Attached Proton Test spectrum indicated the following carbons and their multiplicity; C = 6, CH = 6, CH_2 = 11, CH_3 = 7, 152.10 (q), 110.28 (CH), 56.97 (CH), 52.02 (CH), 39.66 (CH_2), 35.67 (CH_2), 36.85 (q), 177.70 (CO^-), 16.26(CH_3), 15.15 (CH_3), 16.72 (CH_3). HSQC of M_2: H-5 (0.71) # C-5 (56.97), H-18 (1.43) # C-18 (49.68), H-3 (3.16) # C-3 (79.83), H-19 (2.43) # C-19(48.20), H-29a and H-29b (4.69, 4.58) # C-29 (110.3). HMBC of M_2: H-30 (1.68) # C-20(152.10),C-29 (110.3), and C-19 (48.20), H-25(0.89) # C-5 (56.97), C-9 (52.02) and C-4 (38.43), H-29a (4.69) # C-19 (48.20), C-30 (19.60), H-

28 (0.83) # C-18 (49.68), C-16 (35.67) and C-17 (42.20), H-23 (0.96) # C-3 (79.8), C-5 (56.97), C-4 (38.43), and C-24 (16.00), H-24 (0.79) # C-3 (79.82), C-5 (56.97), C-4 (38.43) and C-2 (26.64) .

^{1}H-^{1}H COSY of M_2: H-3 (3.16) # H-2 (1.65), H-29a (4.69) # H-29b (4.58), H-19 (2.43) # H-22 (1.37), H-29a (4.69) # H-30 (1.68), H-29b (4.58) # H-30 (1.68).

Table 1: ^{13}C-NMR and ^{1}H-NMR data of M_2 and Lupeol from literature

Position	δ^{1}H	δ ^{13}C	δ^{1}H	δ ^{13}C
		(M_2);	**(Suryati *et al.*, 2011)**	
1				38.9
2	1.65	26.64	1.62	27.6
3	3.16	79.82	3.18(1H, t)	79.2
4		38.43		39.0
5	0.71	56.97	0.67 (1H, t)	55.5
6	1.53	18.55	1.38 (2H, m)	18.5
7		-		34.4
8		40.22		41.0
9		52.02	1.25 (1H, t)	50.6
10		36.85		37.3
11		19.69		21.1
12		-		25.3
13		-		38.2
14		41.19		43.0
15	1.10	28.19		27.7
16		35.67		35.8
17		42.20		43.2
18	1.43	49.68	1.35 (1H, dd)	48.5
19	2.43	48.20	2.36 (1H, m)	48.2
20		152.10		151.2
21	1.05	28.76		30.0
22	1.19, 1.37	39.66		40.2
23	0.96	28.78	0.96 (3H, s)	28.2
24	0.79	16.00	0.75 (3H, s)	15.6
25	0.89	16.86	0.82 (3H, s)	16.2
26	1.13	16.72	1.02 (3H, s)	16.3
27	1.01	15.15	0.94 (3H, s)	14.7
28	0.83	16.86	0.78 (3H, s)	18.1
29	a. 4.69	110.28	a. 4.68 (1H, d)	109.5
	b.4.58		b. 4.56 (1H, d)	
30	1.68	19.60	1.67 (3H, s)	19.5
CH_3COO^-		31.03		
CO^-		177.70		

The positive Liebermann Buchard test given by compound M_2 suggests that the compound is a terpernoid. M_2 is a white powder with a melting point of 214^{0}C which is the characteristic colour and melting point of pentacyclictriterpene.IR spectrum of the isolated compound showed an intensively broad band at 3433cm-1 for O – H bond vibration of the hydroxyl group. A weakly intense band was seen around 1663 for C=C vibrations. The methylenic part vibration was shown by the band at 2356. The C – H out of plane vibration of the unsaturated part was observed at 889cm $^{-1}$. Corresponding C – C vibration was seen as a weak intense band at 1035 cm-^{1}.The ^{1}H and ^{13}C-NMR spectra of the isolated compound showed a characteristic pattern of a triterpenoid, comparison of the spectra data of M_2 and that of lupeol from literature are similar. In the proton NMR spectrum of M_2, olefinic protons of H-29 (110.28)showed signals at δH 4.69ppm (1H, d) and 4.58 respectively, this supported the double bond between methylene carbon(C– 29) and quaternary carbon (C – 20). H-3 proton appeared as triplet at 3.16ppm. Seven methyl protons also appeared at 1.68, 1.13, 1.01, 0.96, 0.89, 0.83 and 0.79 (3H each, s, CH_3).The ^{13}C-NMR spectrum of the isolated compound revealed the presence of 30 carbon atoms which were further classified into 7 methyls at positions C – 23, C – 24, C – 25, C – 26, C – 27, 28 and C - 30, 11 methylenes at C - 1, C – 2, C – 6, C – 7, C – 11, C - 12, C – 15, C – 16, C – 21, C – 22 and C – 29, 6 methines at C – 3, C – 5, C – 9, C – 13, C – 18, C - 19 and 6 quaternary carbon atoms at C – 4, C – 8, C – 10, C – 14, C – 17 and C - 20 by the APT spectrum of the isolated compound. The HSQC correlation shows the correlation of protons to their corresponding carbons atoms. The proton at δ 0.71 (H - 5) which is a methine proton is correlating with the carbon at δ 56.97 (C - 5) which shows that H – 5 is bounded to C – 12.

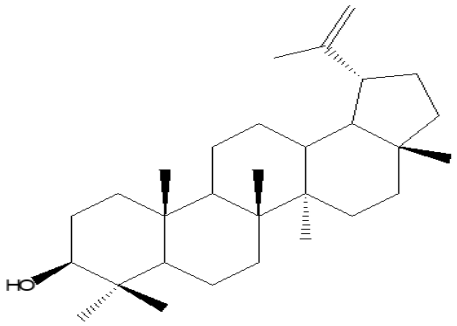

Fig 1: Lupeol ($C_{30}H_{50}O$)

The protons at δ 4.69 and δ 4.58 which methylene protons at position H – 29a and H – 29b respectively is correlating with the carbon at δ 110. 3 (C – 29) which shows that H – 29a and H – 29b are bounded to C – 29. The proton signal at δ 3.16 (H - 3) correlated with methine carbon at δ 79.82 ppm (C - 3) reveals that it belongs to methyneoxy proton C – 3. Long range correlation ^{1}H ^{13}C of HMBC spectrum was used to prove bonding structure relationship of the isolated compound. Long range correlation of proton at δ 1.68 (H - 30) with quaternary carbon at δ 152.10 (C - 20), methylene carbon at δ 110.3 (C – 29) and methine carbon at δ 48.20 (C - 19) revealed that the methyl carbon atom C – 30 binds to the quaternary carbon C – 20. HMBC correlation of methyl protons at δ 0.96 (H - 23) with methine carbons at δ 79.8 (C - 3), δ 56.97 (C - 5), quaternary carbon δ 38.43 (C - 4), methyl carbon at δ 16.00 (C - 24) and the methyl protons at δ 0.79 (C - 24) correlating with δ38.43 quaternary carbon (C – 4), methine carbons at δ 56.9 (C – 5), δ 79.82 (C – 3) and methylene carbon at δ 26.64 (C – 2), supported the dimethyl position at C – 4.Methyneoxy proton H – 3 was coupled by methylene proton H – 2 at 1.65ppm (2H, m) this correlation was established by COSY analysis. The ^{1}H-NMR,^{13}C-NMR, DEPT, HSQC, HMBC and ^{1}H-^{1}H COSY spectral data and comparison with the data described in literature (Suryati *et al.,* 2011) showed the structure of M_2 to be a lupeol (Figure 1). In conclusion, lupeol was isolated from the stem bark of *Leptadenia hastata*. The presence of lupeol in the root extract of this plant may be responsible for the observed ethnomedicinal uses of the plant since various pharmacological activities of lupeol have been reported in literature.

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