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

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**ABSTRACT:** Dried stem bark powder of Leptadenia 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 lupeol of Leptadenia hastata (Pers.) Decne. The structures were established as quercetin 3-O-galacturonopyranoside, quercetin 7-O-α-arabinopyranoside, tamarixin 3-O-galacturonopyranoside, kaempferol 3-O-galacturonopyranoside, 8-hydroxyqueretin 3-O-galactopyranoside, tamarixin 3-Oα-rhamnopyranoside, and tamarixin 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, antimicrobial, anti-protozoal, anti-proliferative, anti-

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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 Asclepiadaceae, (Meve and Liede, 2004). Asclepiadaceae plants are widely used in traditional medicine and have been reported to be rich in steroidal glycosides, cardenolides, flavonoids, triterpenes and polyoxypropyranoderivatives (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 polyoxypropylene 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, tamarixin 3-O-galacturonopyranoside, kaempferol 3-O-galacturonopyranoside, 8-hydroxyqueretin 3-O-galactopyranoside, tamarixin 3-O-α-rhamnopyranoside, and tamarixin 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-
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, powdered 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°C to yield the crude stem methanol extract (118 g). A portion methanol extract (92 g) was suspended in distilled water and partitioned exhaustively and successively with n-hexane, chloroform, ethyl acetate and n-butanol to obtain hexane fraction, chloroform fraction, ethyl acetate fraction and n-butanol fraction respectively. The hexane fraction was subjected to column 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/ethyl acetate as gradient mixture solvent systems of increasing polarity. Several eluates (30 ml each) collected were monitored using TLC, and those with similar TLC profile were pooled together as sub-fractions, evaporated at reduced pressure coded D1 – D7.

RESULTS AND DISCUSSION

Solubility Profile, Chemical Tests and Melting point of M2: 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. M2 is a white amorphous solid substance with a melting point of 214°C. Spectral Analysis: IR Vmax cm\(^{-1}\) of M2: The IR spectrum of M2 showed broad absorption bands at 3433 cm\(^{-1}\) for hydrogen bonded OH stretch, for C-H stretch in CH\(_2\) and CH\(_3\) at 2942 cm\(^{-1}\) 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 C= at 889 cm\(^{-1}\). 1HNMR (500 MHz, Deuterated chloroform) of M2: The proton spectrum of M2 showed signals at 6 4.69 ppm, 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, H), 0.93 (H-23, s, 3H), 0.75 (H-5, s, 1H), 1.68 (H-30, s, 3H) (Table 1). 13C-NMR of M2: The 13C-NMR spectrum of M2 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 M2: TheAttached 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\(_3\)), 36.85 (q), 177.70 (CO), 16.26(CH\(_5\)), 15.15 (CH\(_3\)), 16.72 (CH\(_3\)). HSQC of M2: 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 M2: 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-
The positive Liebermann Buchard test given by compound M₂ suggests that the compound is a triterpenoid. M₂ is a white powder with a melting point of 214°C which is the characteristic colour and melting point of pentacyclictriterpenene. IR spectrum of the isolated compound showed an intensively broad band at 3433 cm⁻¹ for O – H bond vibration of the hydroxyl group. A weakly intense band was seen around 1663 for C=O 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 889 cm⁻¹. Corresponding C – C vibration was seen as a weak intense band at 1035 cm⁻¹. The ¹H and ¹³C-NMR spectra of the isolated compound showed a characteristic pattern of a triterpenoid, comparison of the spectra data of M₂ and that of lupeol from literature are similar. In the proton NMR spectrum of M₂, olefinic protons of H-29 (110.28) showed signals at δH 4.69 ppm (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 3.16 ppm. Seven methyl protons also appeared at 1.68, 1.13, 1.01, 0.96, 0.89, 0.83 and 0.79 (3H each, s, CH₃).The ¹³C-NMR spectrum of the isolated compound revealed the presence of 30 carbon atoms which were further classified into 7 methyls at positions C-20, 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.

Table 1: ¹³C-NMR and ¹H-NMR data of M₂ and Lupeol from literature

| Position | δ¹H | δ¹³C | δ¹H | δ¹³C |
|----------|-----|------|-----|------|
|          |     | (M₂) | (Suryati et al., 2011) |     |
| 1        | 1.65 | 26.64 | 1.62 | 27.6  |
| 2        | 3.17 | 79.8 | 3.18 (1H, t) | 79.2  |
| 3        | 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  |
| CH₃COO⁻ |     | 31.03 |      |      |
| CO⁻      |     | 177.70 |      |      |
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), δ 56.97 (C - 4), methyl proton δ 1.68 (H-13) with methylene 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 - 4), 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 δ 38.43 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. Methylenoxy proton H – 2 was coupled by methylene proton H – 2 at 1.65ppm (2H, m) this correlation was established by COSY analysis. The 1H-NMR, 13C-NMR, DEPT, HSQC, HMBC and 1H-1H COSY spectral data and comparison with the data described in literature (Suryati et al., 2011) showed the structure of M3 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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