# ISOLATION AND CHARACTERIZATION OF STIGMASTEROL GLYCOSIDE FROM THE ROOT BARK OF *Leptadenia hastata*

Supported by tetfund

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Abstract: Preliminary phytochemical screening of the root bark of *Leptadenia hastata* revealed the presence of steroids, flavoniods, saponins, terpenes, glycoside and tannins. Dried root bark powder of *Leptadania hastata* was subjected to cold maceration with methanol to obtain crude methanol extract, which was partitioned with n-hexane, ethylacetate, chloroform and n- butanol to give their respective fractions. Extensive phytochemical screening of the chloroform fraction using column chromatography and gel filtration chromatography resulted to the isolation of a pale white powder of stigmasterol glycoside which was identified by physical test, chemical test, IR, 1D – NMR, 2D – NMR and by comparison with reference spectral data.

**Keywords:** IR, *Leptadenia hastata*, NMR, phytochemical, stigmasterol glycoside

## Introduction

Plant compounds have played important roles in the development of an impressive number of novel synthetic drugs. *Leptadenia hastata* (Pers.) Decne (*L. hastata*) is a perennial specie 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 polyoxypregnane derivatives (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).

*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).

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* (Mailafiya *et al*., 2017).

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).

Stigmasterol and sitosterol are two phytosterols well spread in plants and animals as well as fungi, and have structural similarity to cholesterol. The most important benefit for these two secondary metabolites is their enrolment amongst the health promoting constituents of natural foods which contains them (European Foods Safety Authority, 2008).

The literature survey reveals that there is no report on isolation and elucidation of any steroidal glycoside from the root bark of *L. hastata*. However, the present work reports herein the isolation and identification of stigmasterol-3-O-β-D-glucoside for the first time from chloroform soluble fraction of the methanol root 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 root bark was shade dried, pounded to powder, labelled and stored for use.

### *Extraction and isolation*

The powdered root bark (3.5 kg) was extracted with 90% methanol using cold maceration method for 7 days. The extract was freed from solvent by evaporation *in-vacuo* using rotary evaporator at $40^0$C and yeild a reddish brown residue (75 g) subsequently referred to as the crude methanol extract. Part of root bark methanol extract (65 g) was suspended in distilled water and partitioned successively with n-hexane, chloroform, ethylacetate, and n-butanol to obtain hexane fraction, chlorofom fraction, ethylacetate fraction and *n*-butanol fraction, respectively. The chloroform fraction was then subjected to column chromatography.

### *Column chromatography of chloroform fraction*

The chloroform fraction (7 g) was weighed and subjected to extensive column chromatography; Methanol (5 ml) 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 with hexane (100%), hexane:ethylacetate (9:1) to ethylacetate (100%). Several elutes collected were monitored with TLC using Hexane:Ethyl acetate (9:1, 8.5:2.5, 7:3, 6:4, 1:1), n-hexane (100%),

Ethylacetate (100%), Ethylacetate:hexane (9:1, 8:2, 7:3) as solvent systems. A total of 95 fractions were collected and merged based on their TLC profile giving 30 major fractions coded C1 – C30.

Fraction C18 was subjected to gel filtration using sephadex LH-20. Two (2) ml each of a total of 55 collections were made and combined based on their TLC profile to afford 15 major fractions B1 – B15. Fraction B5 – B14 were further purified by gel filtration repeatedly using sephadex LH-20 led to the isolation of Compound $M_3$. The TLC of $M_3$ was carried out using *n* - hexane: Ethylacetate (8:2), *n*-hexane:Ethylacetate (1:9) as solvent systems with Rf of 0.56, viewed under normal day light and sprayed with 10% sulphuric acid respectively, revealed homogenous spot. $M_3$ gave a positive test to Liebermann Buchard and Salkowski reagents for steroidal nucleus (Kandati *et al.*, 2012; Victor and Chidi, 2009).

$M_3$ was subjected to IR, $^1$H-NMR, $^{13}$C-NMR and 2D-NMR to ascertain the chemical structure.

**Results and Discussion**

***Solubility profile, chemical tests and melting point of $M_3$***

The compound gave a pink single spot on the TLC plate with Rf value of 0.56, it was soluble in methanol, and a positive Liebermann Buchard and Salkowski test for steroidal nucleus, giving an indication that the compound is of steroid base. $M_3$ is a pale white powdery substance with a melting point of $279^0$C which is the characteristic colour and melting point for steroid.

***Spectral analysis***

The IR spectrum showed a strong absorption band at 3401 and 1032 which were characterized as bands for glycoside compound. The absorption bands at 3365 $cm^{-1}$ is for –OH stretching, the bands at 2938 and 2867 for aliphatic groups, 1636 for C=C, the bands at 1459 and 1380 are for $–CH_2$ and $CH_3$, respectively, C – O at 1263, 1172, 1065, 1030.

The proton NMR spectrum of $M_3$ showed peaks from δ 0.68 – δ 5.35 ppm, the proton of H-3 appeared as a multiplet at 3.35 ppm, and revealed the existence of signals for olefinic proton at δ 5.07 (H - 5), 5.35 (H - 6), 5.17 (H - 22) and 5.04 (H - 23). Angular methyl protons were seen at δ 0.68 (s) and 1.00 (s) corresponding to C-18 and C-19 respectively. And one anomeric proton observed at δ4.22 (Bai *et al.*, 2005).

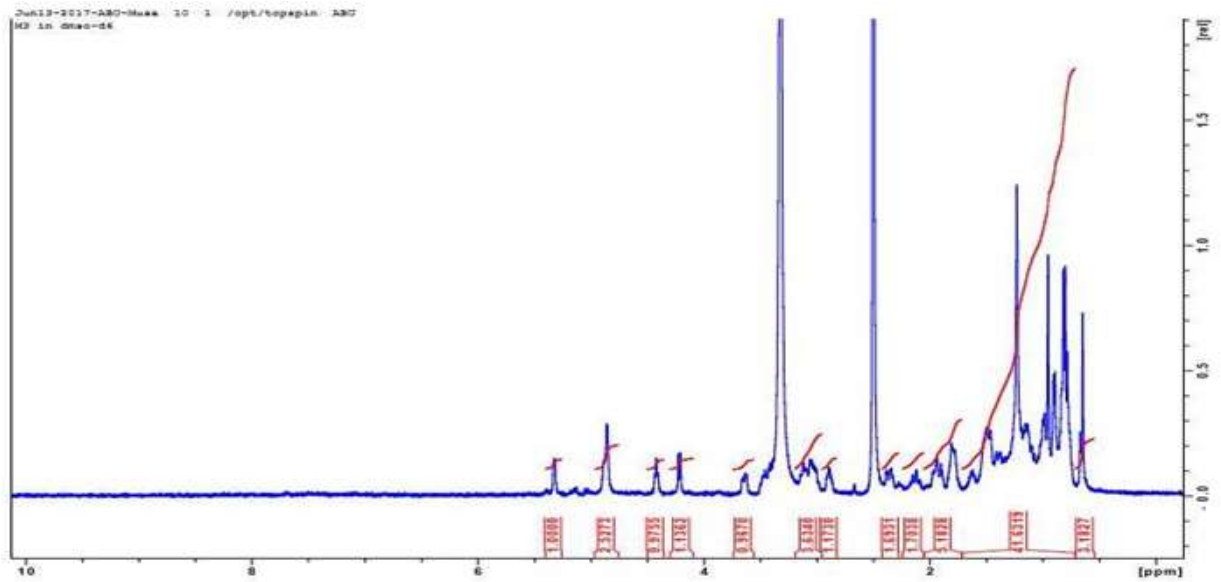



**Fig. 1: $^1$H-NMR of $M_3$**

The proton NMR spectrum of $M_3$ showed other peaks at δ0.68 (H-18), 1.00 (H -19), 0.95 (H-1), 1.01 (H-4), 1.63 (H-16), 2.32 (H-20), 3.37 (H-2′), etc. (Table 1). The $^{13}$C-NMR spectrum of $M_3$ revealed the presence of 35 carbon atom signals in the molecule at 37.31, 31.86, 70.64, 42.35, 140.97, 121.68, 56.66, 35.95, 38.80, 77.27 etc. (Table 1).

The carbon atom at δ101.28 which is an anomeric carbon indicated the presence of a single monosaccharide moiety. The methylene resonance at δ61.61 as well as the four methine resonances at δ74.89, 73.97, 77.22, 70.64 were due to C – 6′, C – 5′, C – 4′ , C – 3′, C – 2′ and C – 1′ respectively of the β – D – glucopyranoside. The signal at δ140.97 corresponded to the C – 5 quaternary carbon of sterol moiety and olefinic resonances at δ121.68, 138.30 and 129.33 corresponded to C – 6, C – 22 and C – 23, respectively. The *J* value of 7.7 on C – 1′ (anomeric proton) reflected that the proton is axial to C – 2′ proton which means glucopyranoside moiety binds to the sterol moiety at β position **(**Bai *et al.*, 2005; Silverstein *et al.*, 1991).

The DEPT indicated the following carbons and their multiplicity; C = 3, CH = 16, $CH_2$ = 10, $CH_3$ = 6. Quaternary carbon at δ140.97 (q), 121.68 (CH), 56.66 (CH), 51.06 (CH), 37.31 ($CH_2$), 38.80 ($CH_2$), 42.35 (q), 77.22 (CH-OH), 19.43 ($CH_3$), 12.30 ($CH_3$), 19.58 ($CH_3$) among others (Figs. 2 and 3).

**Table 1: $^{13}$C-NMR and $^{1}$H-NMR data of $M_3$ and Stigmasterol glycoside from literature**

| Position | δ$^{1}$H | δ $^{13}$C | δ$^{1}$H (Ridhay *et al.*, 2011) | δ $^{13}$C (Ridhay *et al.*, 2011) |
|---|---|---|---|---|
| 1 | 2.36 (2H, m) | 38.28 | 2.36 (m) & 2.13 (m) | 38.28 |
| 2 | - | - | 1.30 (m) | 33.33 |
| 3 | 3. 43 (1H, m) | 77.44 | 3.42 (m) | 76.95 |
| 4 | 1.07 | 39.73 | 1.80 (br, d) | 36.76 |
| 5 | 5.07 | 140.97 | - | 140.43 |
| 6 | 5.35br s | 121.68 | 5.32 (br, d) | 121.04 |
| 7 | - | 31.79 | 1.46 (m) | 31.30 |
| 8 | 1.50 | 31.74 | 1.51 (br, s) | 31.38 |
| 9 | 0.93 (br, s) | 50.10 | 0.99 (br, s) | 49.57 |
| 10 | 1.32 | 35.95 | - | 36.15 |
| 11 | 1.22 (2H, m) | 21.58 | 1.17 | 22.59 |
| 12 | 1.98 | 39.87 | 1.94 (m) & 1.13 (m) | 41.69 |
| 13 | 0.95 | 45.65 | - | 41.80 |
| 14 | 1.05 (1H, m) | 55.93 | 1.08 (m) | 56.20 |
| 15 | 1.03 (2H, m) | 24.34 | 1.12 (m) | 24.72 |
| 16 | 1.63 (2H, m) | 29.15 | 1.91 (br, s) & 1.77 (br, s) | 29.20 |
| 17 | 1.01 (1H, m) | 56.66 | 1.01(m) | 56.11 |
| 18 | 0.68 (3H, s) | 12.30 | 0.65 (s) | 11.76 |
| 19 | 0.98 (3H, s) | 19.58 | 0.99 (s) | 18.99 |
| 20 | 2.32 | 33.85 | 1.34 (m) | 35.37 |
| 21 | 0.91 | 21.39 | 0.91 (d) | 18.76 |
| 22 | 5.17 | 138.30 | 5.18 (dd) | 137.85 |
| 23 | 5.04 | 129.33 | 5.04 (dd) | 128.79 |
| 24 | - | 51.06 | 0.99 (br, s) | - |
| 25 | 1.88 | 31.92 | 1.63 (m) | 31.20 |
| 26 | 0.85 | 20.19 | 0.84 (d) | 19.28 |
| 27 | 0.81 | 19.43 | 0.80 (d) | 18.89 |
| 28 | 1.13 | 25.32 | 1.01 (br, s) | 23.76 |
| 29 | 0.65 | 12.58 | 0.79 (d) | 11.58 |
| 1′ | 4.22 (d) | 101.28 | 4.23 (d) | 100.77 |
| 2′ | 2.93 m | 73.97 | 2.91(m) | 70.14 |
| 3′ | 3.06 (m) | 77.27 | 3.15(m) | 76.75 |
| 4′ | 3.37 (m) | 70.64 | 3.08 (m) | 73.43 |
| 5′ | 3.15 (m) | 77.22 | 3.04 (m) | 76.63 |
| 6′ | 3.40 (m) | 61.61 | 3.66 (m) & 3.50 (m) | 61.10 |
| 2′ - OH` | 4.89 | | 4.73 (br, s) | |
| 3′ - OH | 5.20 | | 4.75 (br, s) | |
| 4′ - OH | 4.82 | | 4.71 (br, s) | |
| 6′ - OH | 4.30 | | 4.30 (t) | |

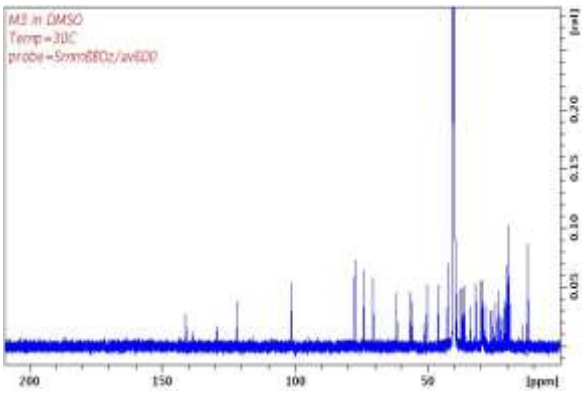



**Fig. 2: $^{13}$C-NMR of $M_3$**

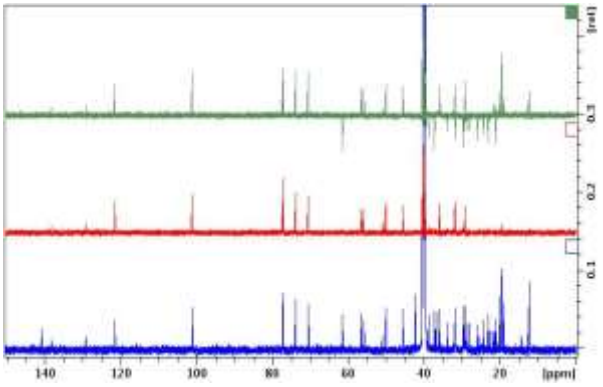



**Fig. 3: DEPT NMR of $M_3$**

The HSQC shows the correlation of protons to their respective carbon atoms. The proton at δ1.98 (H - 12) is correlating with carbon at δ39.87 (C - 12) which shows that H-12 is bounded to C-12. Correlation established by the proton at δ1.03 (H - 15) with the carbon at δ 24.34 (C - 15) shows that H – 15 binds to C – 15. Other proton to respective carbon correlation is seen as follows:

H-16 (1.63) # C-16 (29.15), H-29 (0.68) # C-29 (12.58), H-26 (0.84) # C-26 (20.19), etc. (Fig. 4). Bonding structure relationship was proven through long range correlation $^{1}H \rightarrow {}^{13}C$ of HMBC spectrum. Existence of long range of correlations of protons at δ 4.22 with a carbon at δ 77.22 (C - 3′) and δ 77.27 (C - 5′) indicates that the group of glucose is bounded to C – 3 (oxy carbon $sp^3$).

Correlation between proton at δ 1.01 (H - 17) with methine carbon at δ 37.31 (C - 20) showed that the methine carbon (C -17) binds to the methine carbon C – 20. Long range of correlations between protons at δ 0.68 (H – 18) with a methylene carbon at δ38.80 (C - 12), quaternary carbon at δ 42.35 (C - 13), methine carbons at δ55.93 and 56.66 (C – 14 and C - 15) shows that the methyl carbon (C - 18) binds to the quaternary carbon (C – 13). Then long range of correlations of protons at δ 0.98 (H - 19) with quaternary carbons at δ 35.95 (C – 10), methine carbon at δ50.10 (C – 9) and quaternary carbon at δ140.97 (C - 5) revealed that the methyl carbon C – 19 binds to the quaternary carbon C – 10. Correlations were established between proton doublet at δ 0.91 (H - 21) with methine carbons at δ 55.93 and 33.85 (C – 17 and C – 20 respectively) this shows that the methyl carbon at C – 21 binds on methine carbon at C– 20. In addition proton doublet at δ0.85 (H - 26) establish correlation with methine carbon at δ31.92 (C - 25) and methyl carbon at δ19.43 (C - 27) showed that the methyl carbon C – 26 bind to the methine carbon at C – 25. Other correlations established were: H-9 (0.93) # C-19 (19.43), C-10 (35.95) and C-14 (55.93); OH - 4′ (0.82) # C - 6′ (61.61), C - 2′ (70.64), C - 4′ (73.97) and C - 1′ (101.28); H - 1′ (4.22) # C - 2′ (73.97); H-18 (0.68) # C-14 (55.93), C-13 (42.25), C – 12 (38.80) and C-17 (56.60), etc. (Fig. 5).

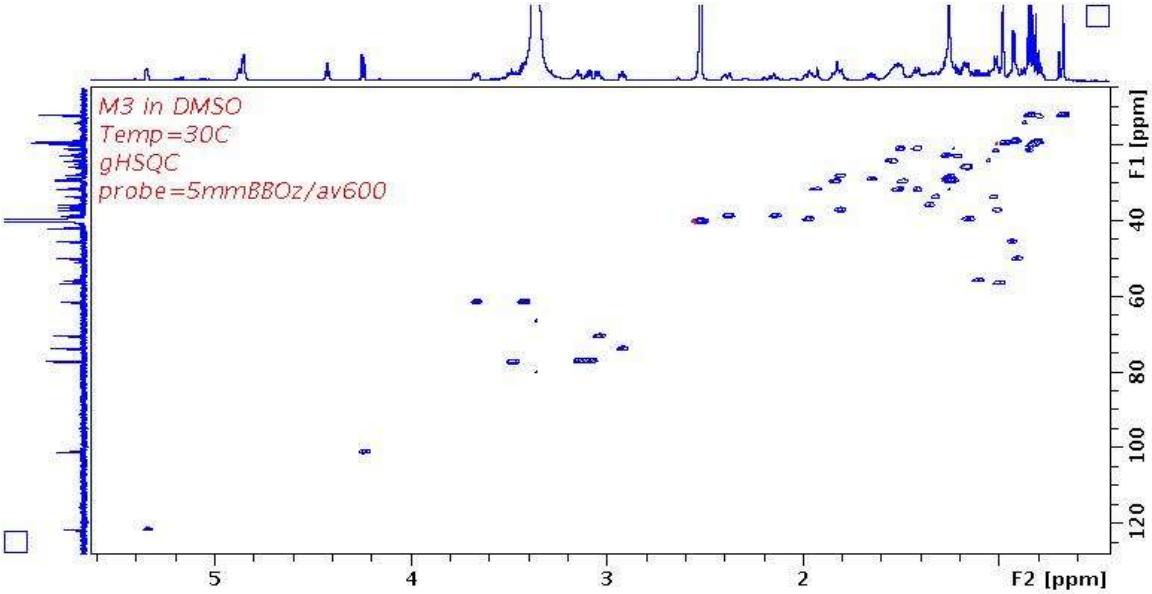



**Fig. 4: HSQC of $M_3$**

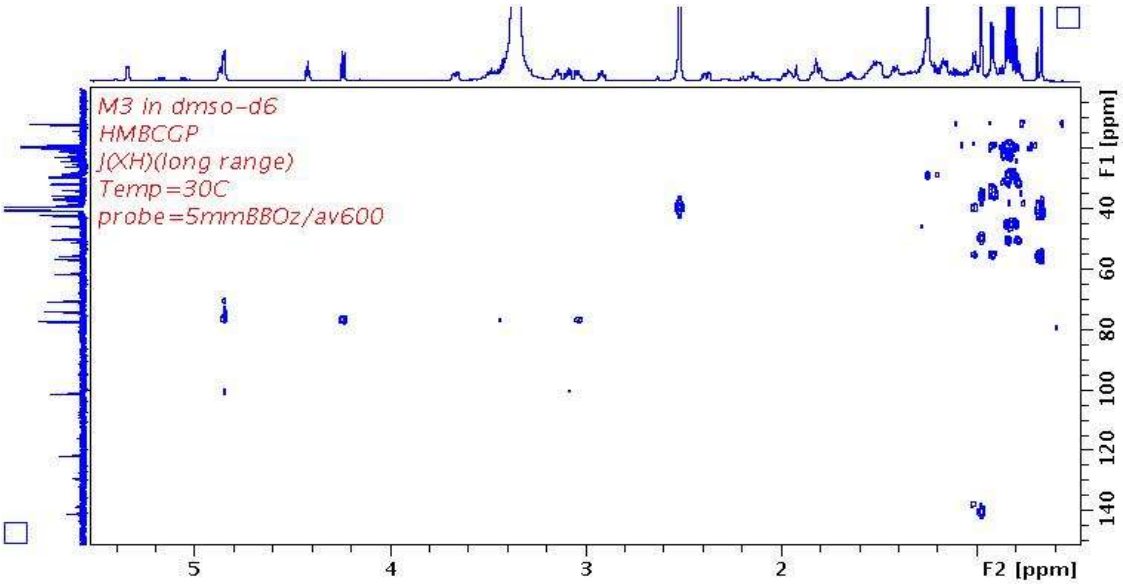



**Fig. 5: HMBC of $M_3$**

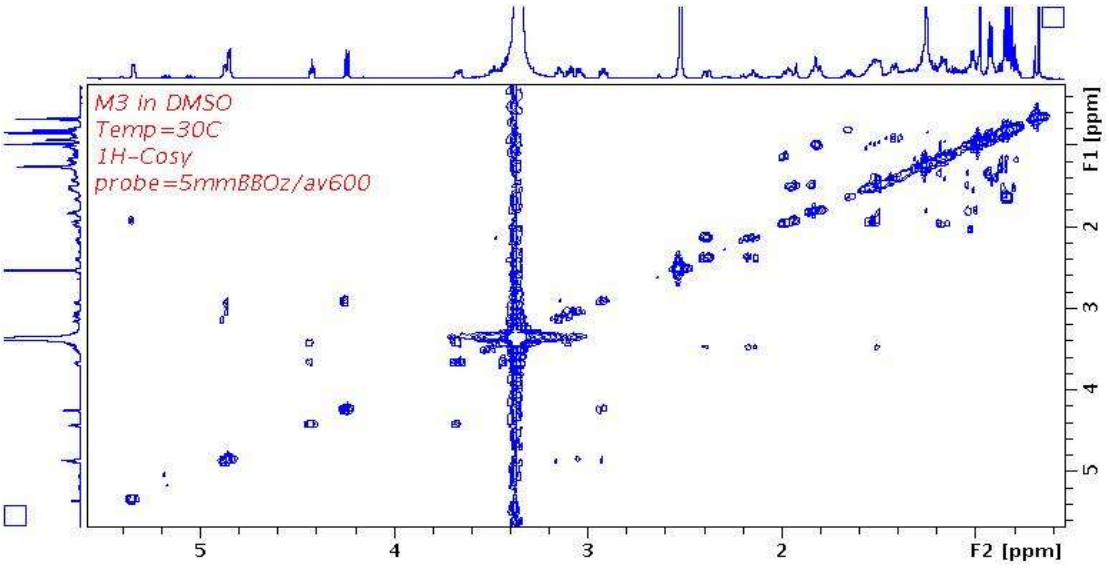
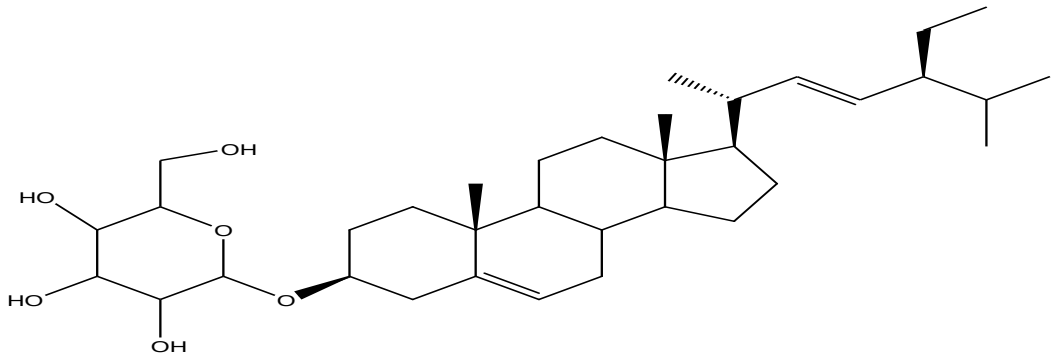



**Fig. 6: $^1H$–$^1H$ COSY of $M_3$**

**$M_3$**




$C_{35}H_{58}O_6$

**Schedule 1: Stigmast - 5, 22-dien-3-O-β-D-glucopyranoside**

$^1H$ - $^1H$ COSY spectrum of $M_3$ shows a proton correlation with its neighboring proton. The proton at δ 1.22 (H - 11) showed correlation with the proton at δ 1.98 (H - 12) and proton at δ1.63 (H - 16) established correlation with the proton at δ1.01 (H - 17) (Fig. 6). The $^1H$-NMR, $^{13}C$-NMR, DEPT, HSQC, HMBC and $^1H$-$^1H$ COSY spectral data and comparison with the data described in literature (Ridhay *et al.*, 2011) showed compound $M_3$ to be a stigmasterol glycoside (Schedule 1).

**Conclusion**

A known steriodial glycoside; stigmasterol glycoside has been isolated from the root bark of *Leptadenia hastata* and its chemical structure elucidated using spectroscopic method.

**Conflict of Interest**

Authors have declared that there is no conflict of interest reported in this work

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