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β-Stigmasterol is present in the stembark of *Lonchocarpus sericeus* Poir. (Papilionaceae)

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Abstract
A phytosterol β-Stigmasterol, was isolated from the hexane fraction of the stembark of *Lonchocarpus sericeus* Poir. (Papilionaceae), a medicinal plant commonly used in folkloric medicine in Southern Nigeria for the treatment of inflammation and pain. Its structure was established by FT-IR, ^{1}HNMR, ^{13}CNMR, 2-dimensional NMR spectroscopy and by direct comparison of data with those in literature.

Keywords: B-Stigmasterol, chromatography, *Lonchocarpus Sericeus*, spectroscopy

Introduction
Lonchocarpus sericeus Poir. (Papilionaceae) is a leguminous plant which is known as Senegal lilac or Cube root. It is a dry deciduous tree that can grow from 10 to 16 meters high and flowers with dense hanging racemes of purple flowers which makes it perfect for display purposes. The flowers have a marked smell similar to vanilla [1, 2]. In Nigeria, leaves are used for general healing while the bark is used for treatment of body pains, arthritis, rheumatism, cutaneous, subcutaneous parasitic infection, convulsions. It is also used as fish-poisons and laxatives. The roots are used for treatment of leprosy. The fruit and seeds are used as insect repellants and arachnicides [3].
There are scanty reports on previous works done on the stembark of the plant unlike other parts of the plant. The available reports include: isolation of a pentacyclic triterpenoid lupeol from the stembark of *L. sericeus* [4] as well as the anticonvulsant activity of methanol extract of the stem bark of *L. sericeus* [5]. Therefore, the present study is aimed at isolation and characterization of more bioactive compounds from the stembark of *L. sericeus*.

2. Materials and Methods
2.1 Plant materials
The fresh stembarks of *L. sericeus* were collected from a forest edge in Ikono Local Government Area of Akwa Ibom State, Nigeria and were identified and authenticated by a botanist Mr. Ndukwe Ibe, of the Department of Forestry, Michael Okpara University of Agriculture, Umudike, Nigeria. The plant sample was further confirmed at the Pharmacognosy and Natural Medicine Department of the University of Uyo, Nigeria, where a voucher specimen (UUH 62/19) of the plant has been deposited in the Herbarium

2.2 Extraction and Partitioning
The plant part (stembarks) were washed and shade-dried for two weeks. The dried stembarks were further chopped into small pieces. The chopped stembarks (2.0 kg) was macerated in 97% methanol for 72 h to give the crude methanol extract. The liquid filtrate was concentrated and evaporated to dryness in *vacuo* at 40 ^{0} C using rotary evaporator. The dried crude extract was stored in a refrigerator at 4 ^{0} C until use for the proposed experiment.
The methanol extract of *L. sericeus* stembark (100 g) was dispersed in 300 ml of distilled water and partitioned into *n*-hexane using a separating funnel. The fraction was subsequently concentrated under reduced pressure in a rotary evaporator (WG-EV311-V, Wilmad-LabGlass, USA) at 40 °C until they became completely dry. The hexane fraction (17.0 g) was stored in a sealed container and kept in a refrigerator at 4 °C until analysis.

2.3 Isolation and purification of compound
The hexane fraction (17.97g) was submitted to silica gel column (540 g, 70-230 mesh) eluted with hexane/DCM/EtOAC gradient, yielding 103 fractions, which were pooled based on similarities in R_f values.

Based on their TLC profiles, similar eluates were combined to yield 18 fractions. They were further combined to yield 9 fractions as follows: A_1-A_{41}, A_{42}-A_{51}, A_{52}-A_{60}, A_{61}-A_{69}, A_{70}-A_{74}, A_{75}-A_{78}, A_{79}-A_{82}, A_{83}-A_{93}, and A_{94}-A_{103}.
Fractions A_{61}-A_{69} (1.86 g,) and A_{70}-A_{74} (0.28 g) (Hex/DCM 10:90) were pooled together and chromatographed on a column using gradient of hexane/DCM/EtOAC to yield 149 fractions (C_1-C_{149}). They were further reduced to 14 fractions (C_1-C_8, C_9-C_{22}, C_{23}-C_{27}, C_{28}-C_{33}, C_{34}-C_{40}, C_{41}-C_{51}, C_{52}-C_{62}, C_{63}-C_{74}, C_{75}-C_{90}, C_{91}-C_{95}, C_{96}-C_{103}, C_{104}-C_{113}, C_{114}-C_{144} and C_{145}-C_{149}). Fractions C_{91}-C_{95} were subjected to preparative TLC on silica gel developed in hexane/DCM (1:6) to afford a pure compound LS_2 (66 mg).

2.4 Spectroscopic characterization

The NMR of pure compounds was carried out on a Bruker AVANCE 400 (operating at 400 MHz for proton and 400 MHz for carbon). It was processed using a Bruker software. NMR spectra were calibrated using solvent signals (^{13}C: $CDCl_3$ δ 77.0 ppm; ^1H: $CHCl_3$ in $CDCl_3$ δ7.26 ppm). Chemical shifts were given in δ (ppm) and coupling constants reported in Hz. Structural assignments were based on the interpretation of the following NMR experiments: ^1H, ^{13}C, ^1H-^{13}C HSQC. Accurate mass was determined on Gas Chromatography/mass spectroscopy (GC/MS) performed on an Agilent 6890N/5973B GCMS system. Infrared spectra were recorded on a 5500 series compact FTIR (Agilent Technologies) instrument. Melting point was determined with a Gallenkamp melting point apparatus and it was not corrected.
Column chromatography was performed using silica gel (70-230 mesh, Sigma Aldrich) in glass columns of varying sizes fitted with Teflon taps. Analytical Thin Layer chromatography (TLC) was performed on pre-coated aluminum sheets with fluorescence (Silica gel $^{60}F_{253}$ 0.2mm thickness, Sigma Aldrich); preparative TLC was done with TLC plates with florescence (Silica gel $^{60}F_{253}$; 1mm thickness). Detection was done with iodine crystals or by visualization under ultraviolet light at wavelengths 254 and 366 nm.

3. Results and Discussion

The compound LS 2 appeared as a white solid substance with melting point 160-163 °C and R_f value 0.69 (EtAc/Hex: 1/3). The molecular weight determination of LS 2 gave an exact mass of 412.370516 which corresponds to the formula $C_{29}H_{48}O$ with six degrees of unsaturation. The GC-MS spectra of LS2 gave a parent molecular ion $[M]^+$ at $m/z = 412$. The base peak at $m/z = 55$ is attributable to the fragment $[C_4H_7]^+$ in the side chain. Other prominent peaks were $m/z = 271$ which corresponds to [M-side chain-2H], $m/z = 255$ which is due to [M- side chain-H_2O]$^+$. This fragmentation patterns are consistent with those of stigmasterol [6].
The FT-IR spectrum of LS2 indicates a broad absorption at 3321 cm^{-1} which is attributable to the O-H bond vibration of a hydroxyl group. The infra-red absorptions at 2983 cm^{-1} and 2942 cm^{-1} are characteristic of the C-H stretching vibrations of a methyl moiety while its bending vibration was found around 1407 cm as an absorption of medium intensity. The corresponding C=C vibrations was shown around 1654 cm^{-1} as weakly intense band. The corresponding C-C vibration was shown as weak intense band at 1026 cm^{-1}.
In the ^{1}HNMR spectrum of LS2, H-3 proton appeared as a triplet of a doublet doublet (tdd) at δ3.53 and H-6 olefinic proton showed a multiplet at δ5.36. Two olefinic protons appeared downfield at δ5.09 (m) and δ4.96 (m) which were in close agreement with the chemical shift values of H-22 and H-23 respectively for β-stigmasterol [7, 8]. The ^{13}C NMR shows some recognizable signals at 140.93 and 121.43 ppm which is assignable to the double bond at C-5 and C-6 [9]. The δ value observed at 71.99 ppm is due to C-3 β-hydroxyl group [10]. Again the signals observed at 21.26 and 12.16 ppm correspond to angular carbon atoms at C-19 and C-18 respectively (Table 1).

Table 1: Major ^{1}H NMR and complete ^{13}C NMR ($CDCl_3$) data for LS 2, δ in ppm and J in Hz.

	Experimental values		Literature values [7, 9]	
Carbon	**δ_C**	**δ_H**	**δ_C**	**δ_H**
1	37.42		37.3	
2	34.10		31.5	
3	71.99	3.53 (1H, tdd)	71.8	3.25 (tdd, J=4.5 MHz)
4	36.33		42.3	
5	140.93		140.8	
6	121.91	5.36 (1H, m)	121.7	5.14 (1H, m)
7	29.11	-	31.9	
8	32.07		31.9	
9	50.30	-	51.2	
10	36.68		36.5	
11	23.23		21.1	
12	39.94		39.7	
13	42.48		42.3	
14	56.93	-	56.9	
15	24.49		24.4	
16	28.43		28.4	
17	56.21	-	56.1	
18	12.04		11.0	
19	21.26	-	21.2	
20	40.70		40.5	
21	25.59		21.2	
22	138.52	5.09 (1H, m)	138.3	4.62 (1H, m)
23	129.43	4.96 (1H, m)	129.3	4.61 (1H, m)
24	45.99		51.2	
25	29.30		31.9	
26	20.01		21.2	
27	19.58		19.0	
28	26.21		25.4	
29	12.16		12.1	

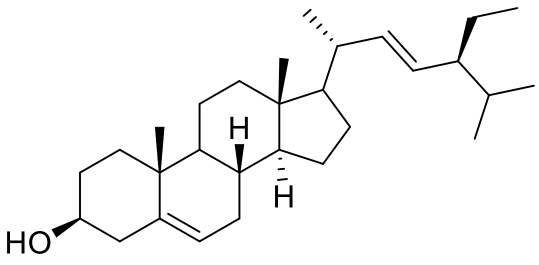

Fig 1: Chemical structure of LS 2 (β-Stigmasterol)

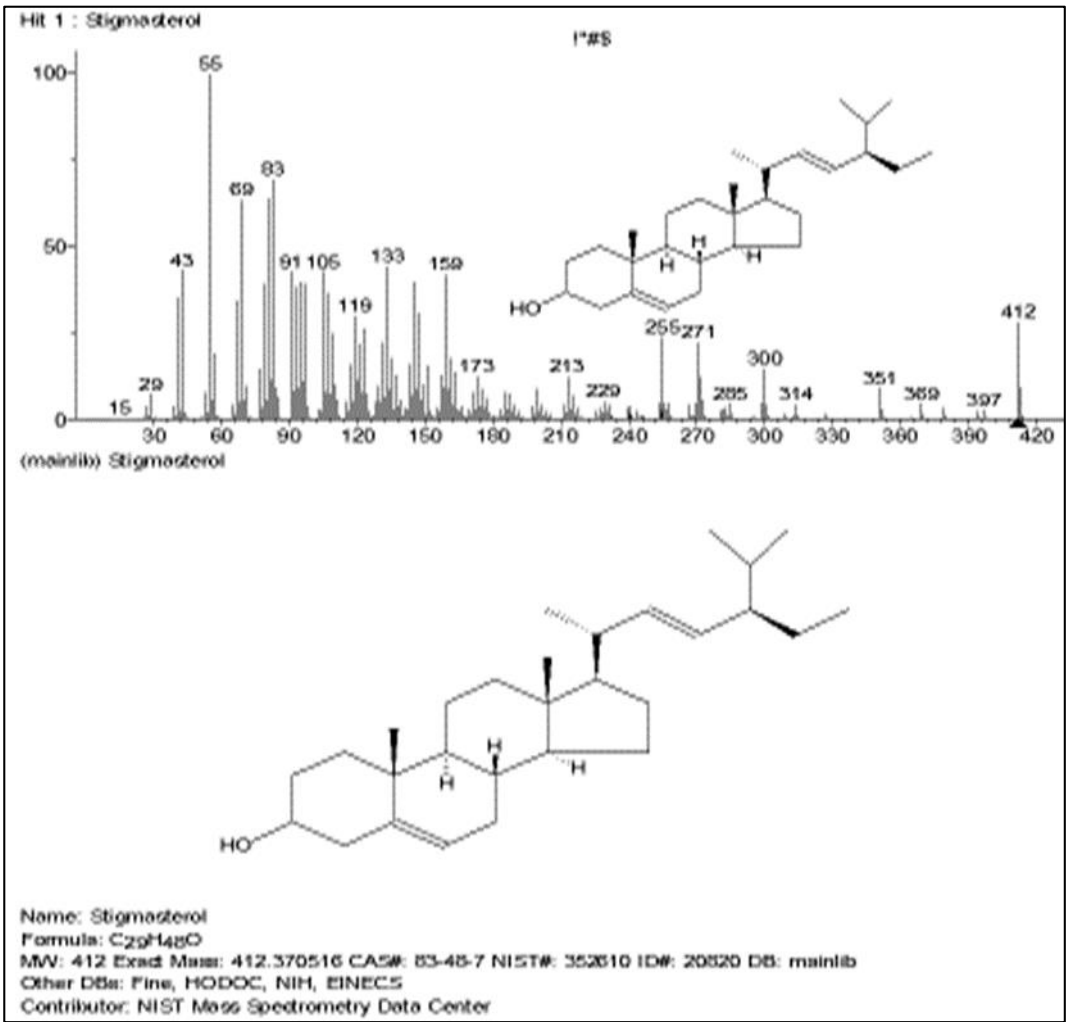

Fig 2: Mass Spectrum of LS 2

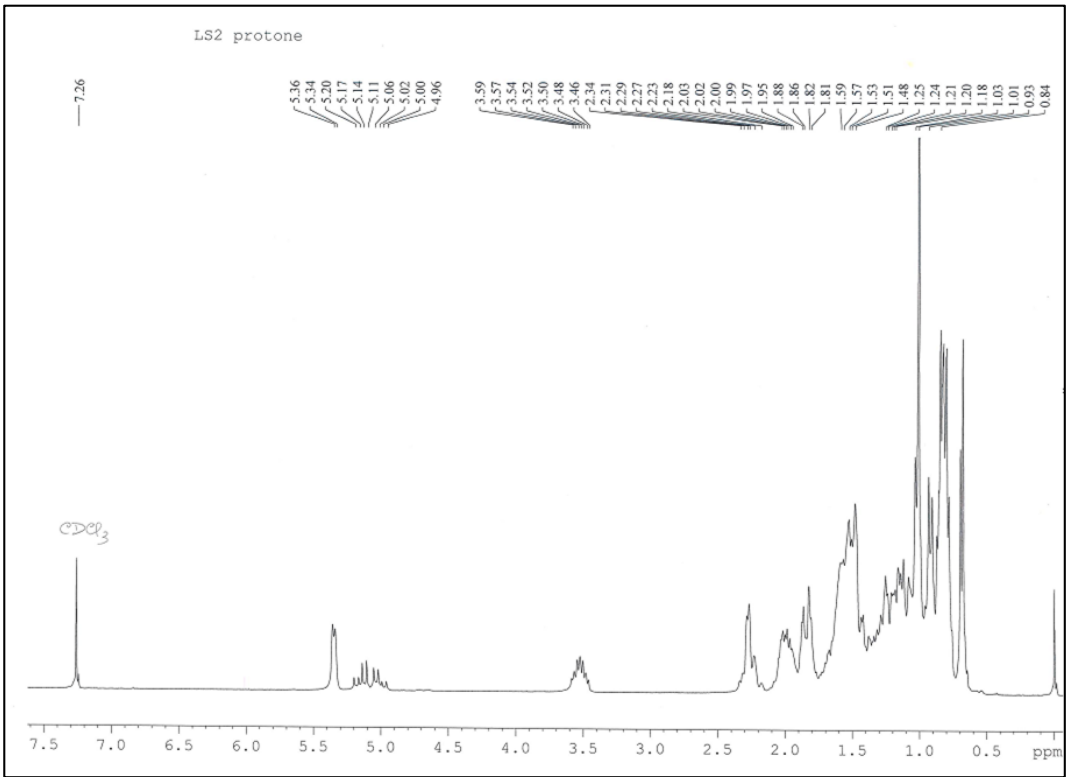

Fig 3: [1]HNMR Spectrum of LS2

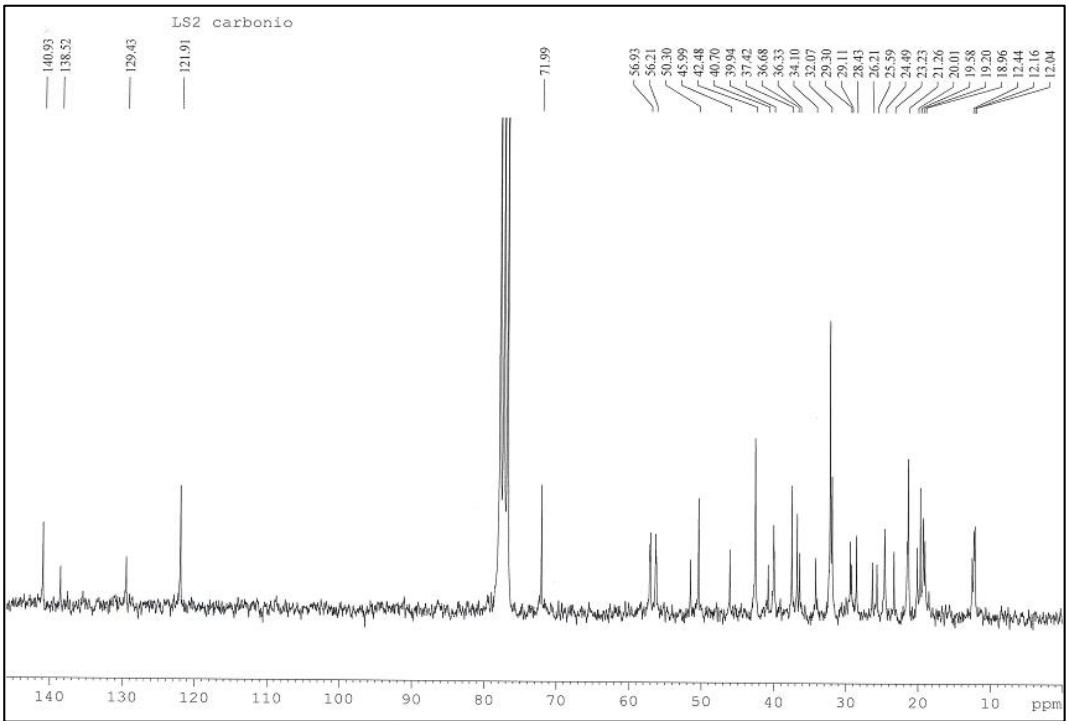

Fig 4: ^{13}CNMR Spectrum of LS2

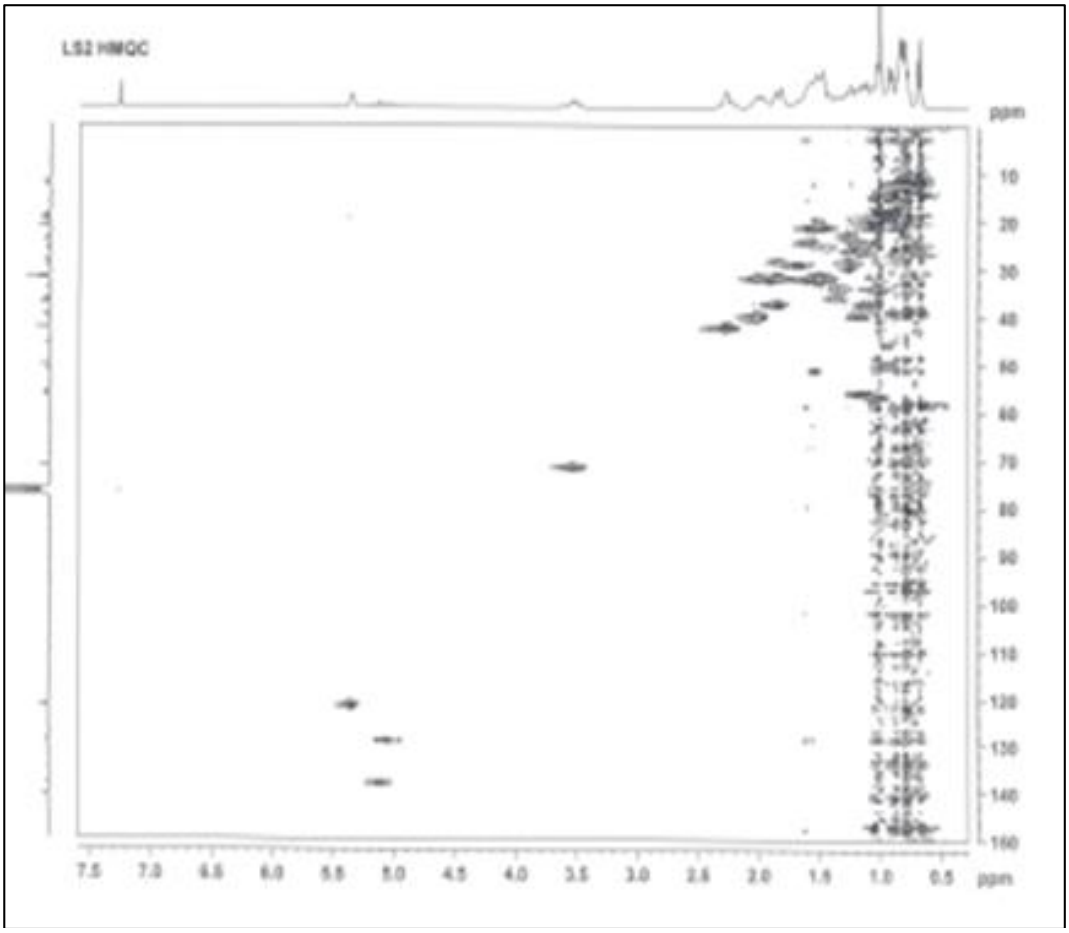

Fig 5: HMQC Spectrum of LS2

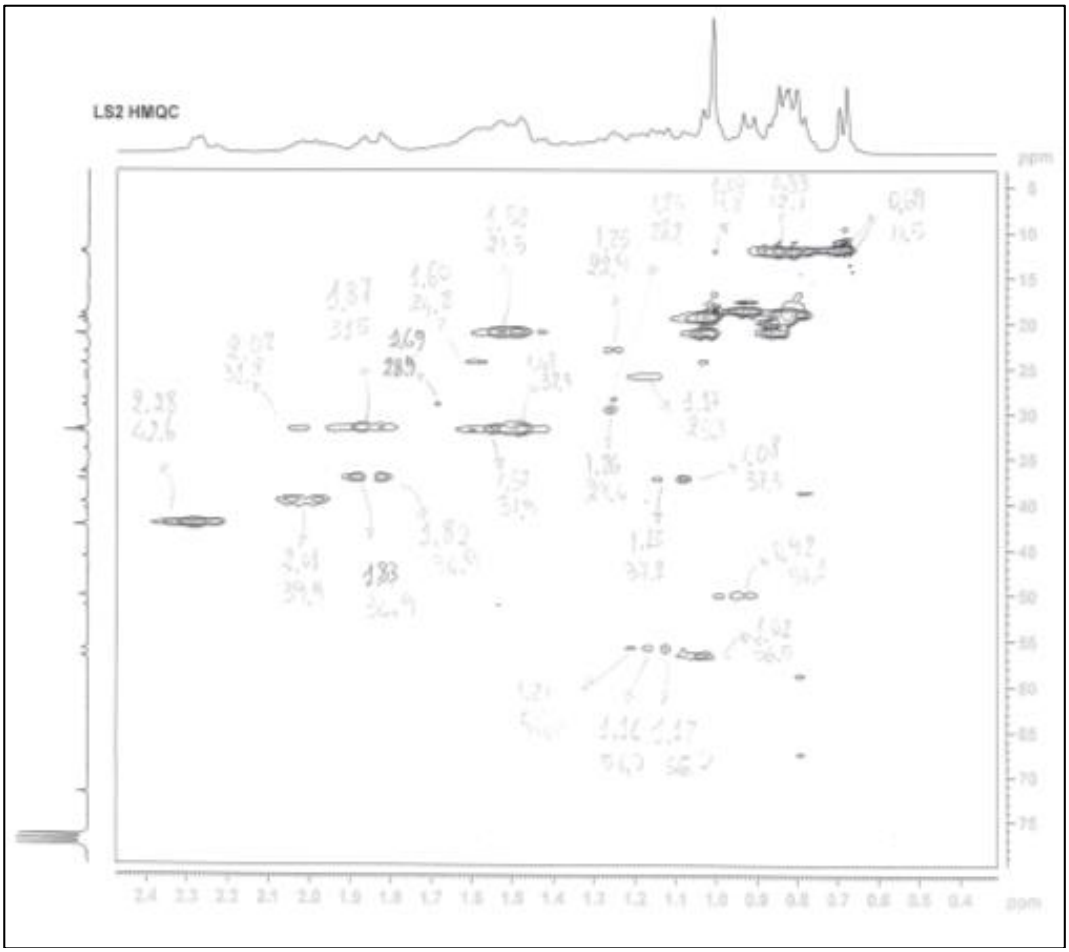

Fig 6: HMQC Spectrum (expansion) of LS2

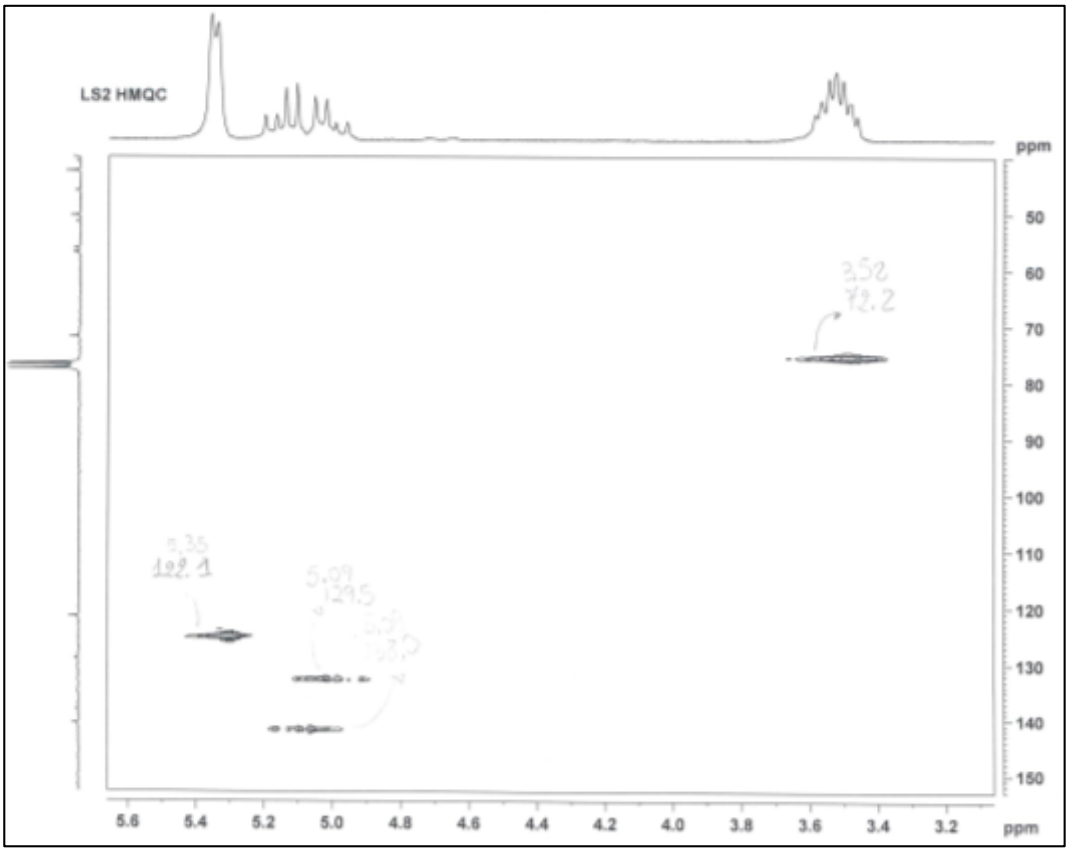

Fig 7: HMQC Spectrum (expansion) of LS2

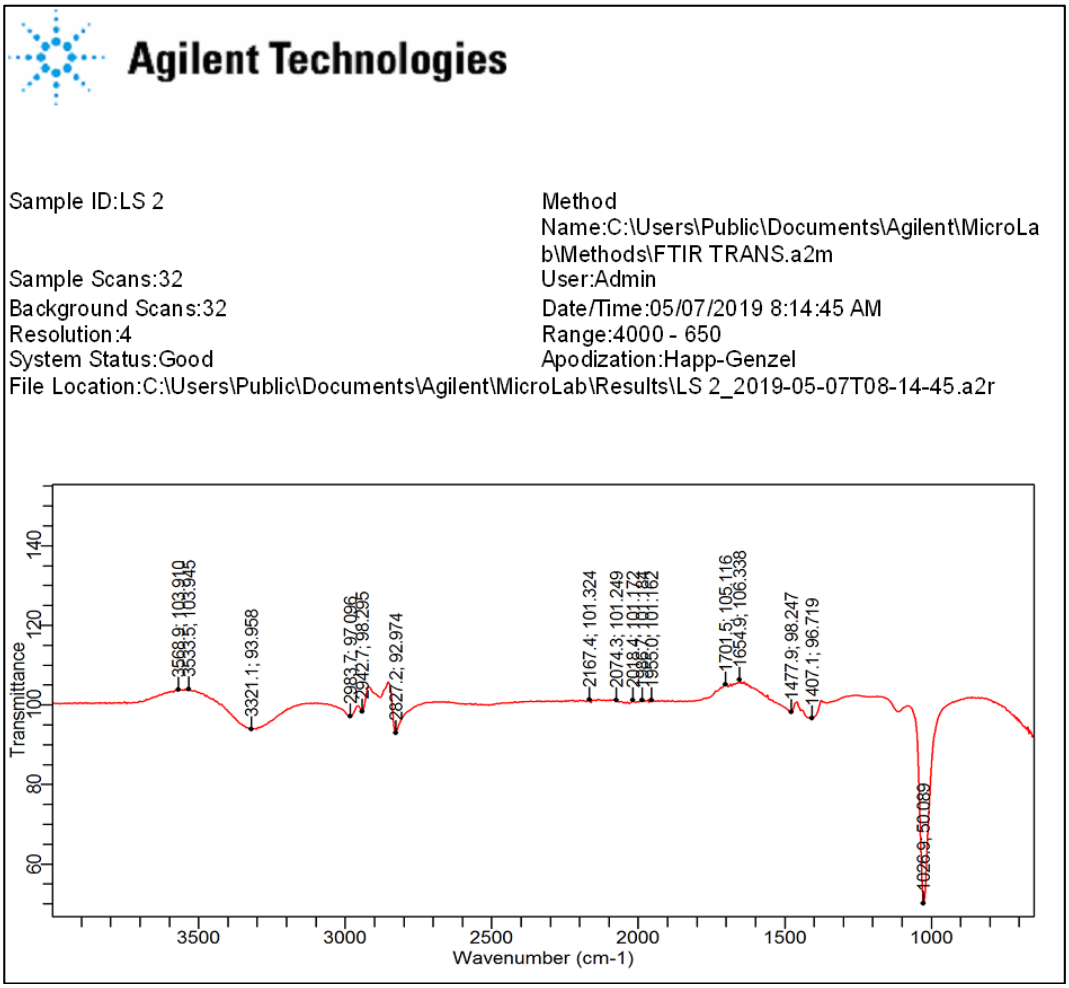

Fig 8: FT-IR Spectrum of LS 2

4. Conclusion

The hexane fraction of the stembark of *Lonchocarpus sericeus* chromatographed on silica gel in hexane/DCM afforded a white solid substance LS 2. Physical and spectral characteristics of LS 2 have confirmed its identity as β-stigmasterol, a well-known phytosterol.

5. Acknowledgement

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6. Conflicts of Interest: Authors declared there are no conflicts of interest to disclose.

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