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Isolation and Structural Characterization of Stigmasterol from the Leaves of *Gossypium hirsutum* (Malvaceae)

[*1]Ahmed, R.J., [1]Sani, Y.M., [1]Abdullahi, S.M. and [1]Jimoh, A.A.

[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

*Corresponding Author: ramatahmed58@gmail.com

ABSTRACT

Stigmasterol, a pentacyclic triterpenoid, was isolated from the hexane fraction of methanol leaves extract of *Gossypium hirsutum*, a plant used in traditional medicine for treatment of malaria, dysentery, hypertension, diabetes, menstrual pain, bacterial infections and cancer. The isolation of the compound was carried out by column chromatography. The compound was characterized using ^{1}HNMR and ^{13}CNMR spectral data as well as comparison with reported data. The compound appeared as white powdery solid which was completely soluble in chloroform with a melting point range of 135-137◦C.

Keywords: *Gossypium hirsutum*, IR, NMR, Stigmasterol,

INTRODUCTION

Gossypium hirsutum belongs to the Malvaceae family a large family of about 200 genera and about 2300 species [1]. It is an annual or a perennial shrub that grows to approximately 1.5 – 2 m in height. Its flowers are cream in colour and the leaves are generally flat. Both parts of the plant trap sunlight to maximize light absorption throughout the day which reduces photo bleaching and transpiration [2]. It is commonly called *auduga* by the Hausas, *ro-afor* by the Igbos, and *igi-ora* by the Yorubas in Nigeria [3]. Its seeds and roots are used in nasal polyps, uterine fibroids and other types of cancer of the body [4]. Flowers are used as diuretic, emollient and in hypochondriasis. Leaves steeped in vinegar is applied to the forehead for headache, root decoction is used for asthma, diarrhea, and dysentery [5]. Root bark is used to stimulate secretion of breast milk among the Yorubas in Nigeria [6].

Literature on phytochemical screening of the leaves of *Gossypium hirsutum* revealed the presence of alkaloids, saponin, cardiac glycoside, tannins, flavonoids, terpenoids, phenolic

compound and steroid [6]. The phytochemical investigation of the plant flowers led to the isolation of kaempferol, quercetin, and hyperoside flavonoids [7]. Diglycosylated flavonol (3-glucoside-7-rhamnoside of 3, 5, 7, 4′-tetrahydroxy -8- methoxyflavone) was also isolated from immature flower buds of *Gossypium hirsutum*. In spite of its varied medicinal potentials, there is paucity of data on the isolation and characterization of any compound from the aerial part of this plant. This study was therefore carried out to extract, isolate and elucidate stigmasterol from the leaves of *Gossypium hirsutum*.

MATERIAL AND METHODS

Plant Collection and Identification

The plant sample comprising the leaves and flower was collected from Lokoja metropolis, in November, 2018. The plant sample was authenticated by Mal. Namadi Sunusi of the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, by comparing with herbarium references. Voucher specimen number 0453 was assigned. The leaves were removed, shade dried, pulverized, labelled and stored for further use.

Preparation of Extract

The powdered leaves (700 g) were extracted with methanol using maceration method with occasional shaking for 72 hours and the solvent was removed using rotary evaporator. The extract (120 g) was suspended in distilled water and filtered using a filter paper to obtain water soluble and water insoluble portions. Both the water soluble and insoluble portion were partitioned with n-hexane, chloroform, ethyl acetate and n-butanol to give the n-hexane, chloroform, ethyl acetate and n-butanol fractions respectively.

Phytochemical Screening

The crude methanol extract was subjected to phytochemical screening for the presence of alkaloids, glycosides, tannins, steroids/triterpenes, saponins and flavonoids according to standard protocol [9].

Column Chromatography of Hexane Fraction

The hexane fraction (7 g) was subjected to column chromatography over a silica gel-packed column of dimension 75 cm x 3.5 cm and was eluted by gradient elution technique with hexane 100% followed by 98:2 hexane:ethyl acetate and increasing the polarity gradually by 5 up to

100% ethyl acetate. Finally, the column was eluted with 100% methanol. Eluents of 100 mL aliquots were collected and monitored on TLC for similar fractions. A total of 123 collections were made and pooled together based on similarity in their TLC profile to give appreciable major column fractions coded A to T [8].

Column fraction I was subjected to further separation over silica gel in a smaller diameter sized column (1 cm x 50 cm). Gradient elution technique was employed; starting with hexane 100% (100 ml) followed by 95:2 DCM: ethyl acetate to ethyl acetate 100% and 100% methanol (2 ml each) which afforded a white powdery compound (5 mg) coded RO. The compound gave a single homogenous spot two different TLC solvent systems: DCM: Ethyl acetate in the ratios 9:1 and 5:1 indicating its purity. The melting point of the isolated compound was determined using gallenkamp melting point apparatus at the Department of Pharmaceutical and Medicinal Chemistry, ABU, Zaria. Compound RO was also subjected to some tests: Liebermann Burchard's test [9] and spectral analysis (1D NMR) using Bruka AVANCE III NMR spectrometer (400 MHz) at the School of Pharmacy University of London to elucidate its chemical structure.

RESULTS AND DISCUSSION

Solubility Profile of Compound RO

Compound RO was completely soluble in chloroform and sparingly soluble in methanol but insoluble in hexane.

Melting point of Compound RO

The sample, compound RO, had an uncorrected melting point range of 135 -137°C.

Chemical Tests on Compound RO

RO produced reddish colour when subjected to Liebermann Burchard's test [9].

Spectral Analysis

IR spectroscopic analysis of RO (Figure 1) showed absorption bands at 3354.6 cm^{-1} typical of OH stretching; 3071 $cm^{-1}$was due to SP^2 C-H stretching vibration of alkene, 2922.2 $cm^{-1}$and 2855.1 cm^{-1} due to CH stretching resulting from asymmetric and symmetric vibrations, 1684.76 cm^{-1} due to C=C stretching vibrations and 1449.9 cm^{-1} due to bending frequency of cyclic

methylene and 1028 cm^{-1} is due to cycloalkane. These results are similar to those reported in the literature [10].

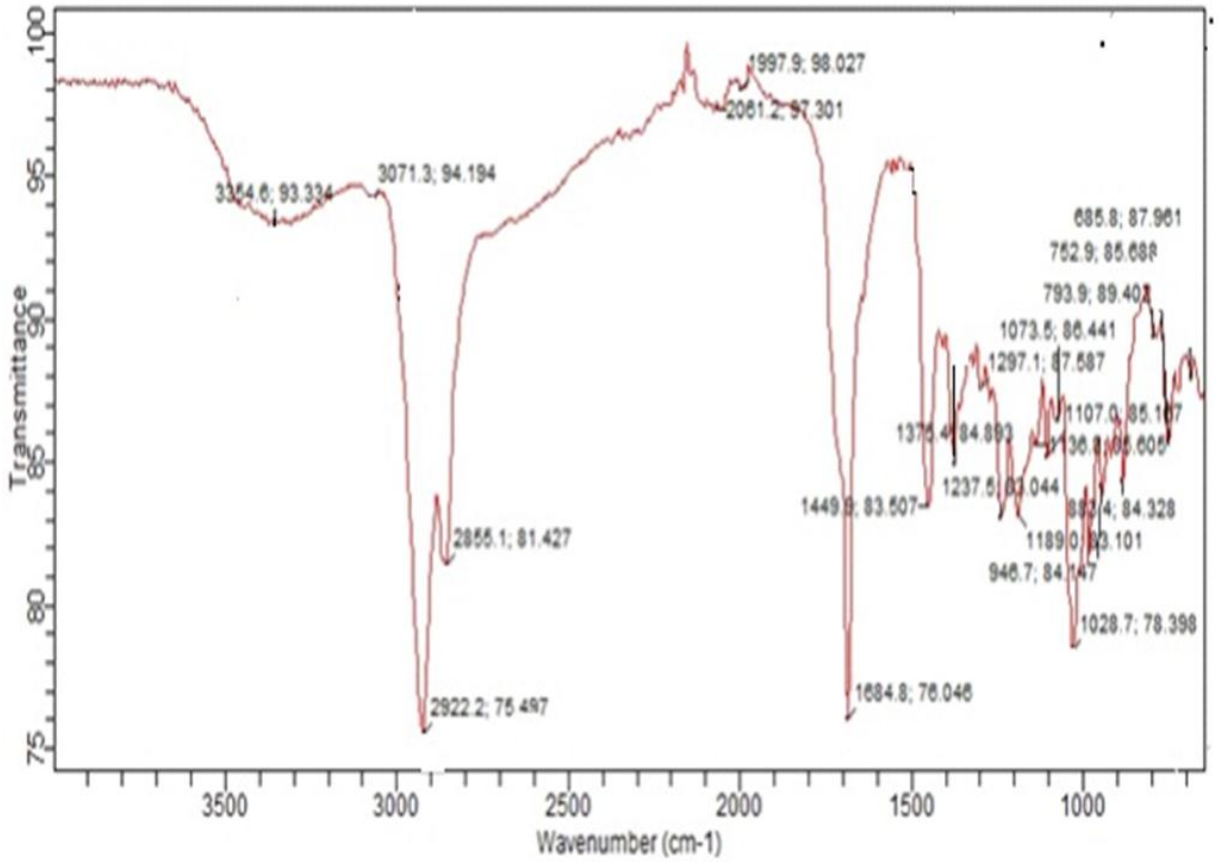

Figure 1: IR spectrum of RO

The ^{1}H NMR spectrum of RO as shown in figure 2 revealed the presence of six high intensity peaks indicating the presence of 6 methyl groups at δH 0.72, 0.82, 0.87, 0.91, 0.94, 1.03. The proton corresponding to the H-3 of a steroid moiety appeared as a multiplet at δ 3.55ppm. At δ 5.02 ppm to δ 5.38 ppm, this corresponds to a peak in the form of a singlet in the region of the ethylene protons suggesting the presence of three protons, (δ 5.05 (1H, s, H-23), 5.17 (1H, s, H-22) and 5.29 (1H, d, H-6)). Angular methyl proton at 1.03 (s) and 2.26 (s) corresponds to C-19 and C-20 proton respectively.

The ^{13}C NMR of RO (figure 3) showed recognizable signals at δ 140.76 and 121.72 which are assigned C5 and C6 double bonds respectively. The values at 19.83 ppm and 39.79 ppm correspond to angular carbon atom (C-18 and C-19), δ 138.31 for C-22 and δ 129.29 for C-23. Spectra show twenty-nine carbon signal including six methyls, nine methylenes, eleven

methane and three quaternary carbons. The olefinic carbons appeared at δ140.76(C-5), 138.31 (C-22), 121.72 (C-6), 129.29 (C-23) and the carbon attached to the OH group at 71.00 (C-3).

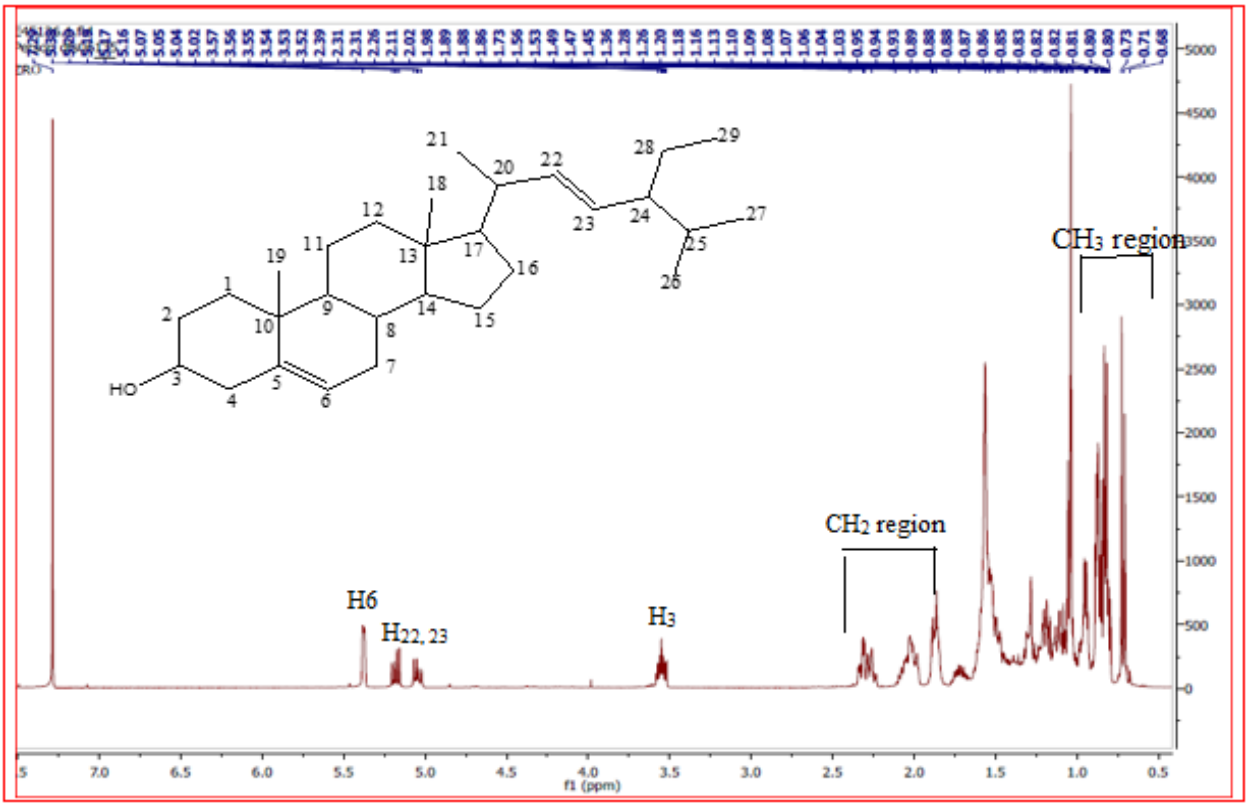

Figure 2: 1H NMR spectrum of compound RO

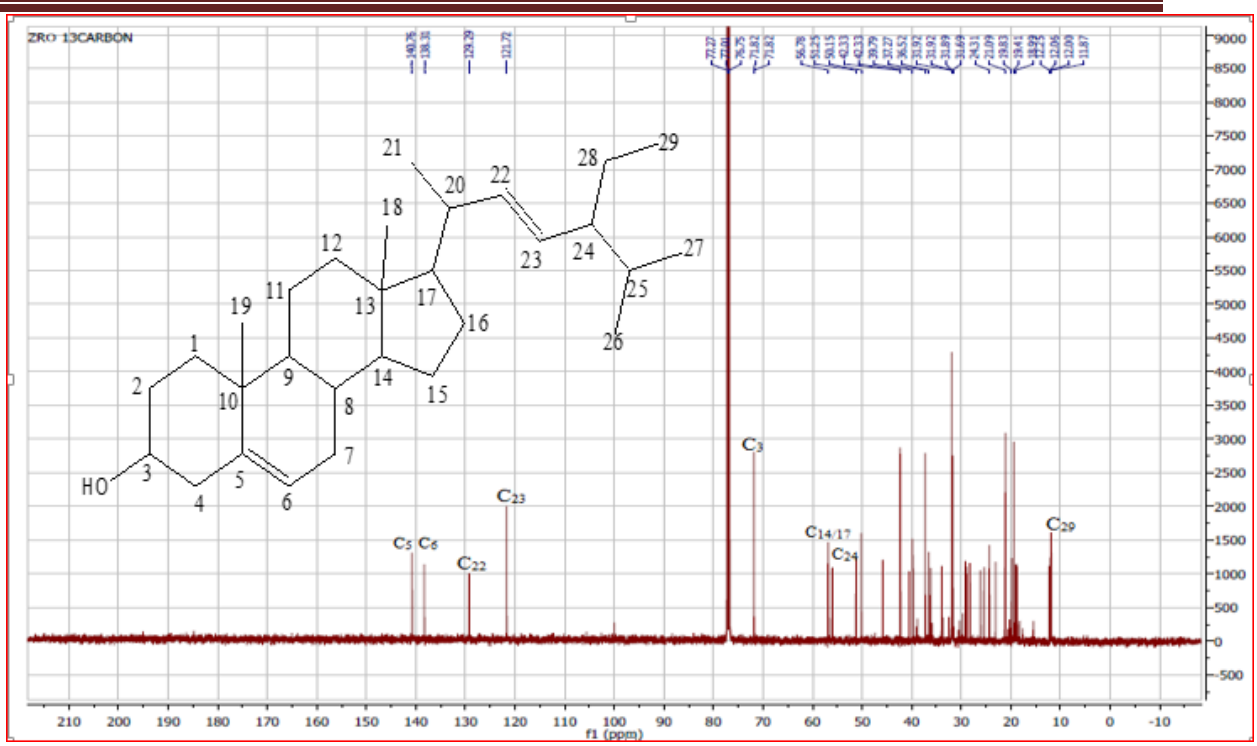

Figure 3: 13C NMR spectrum of compound RO

Based on the aforementioned data, compound RO was confirmed to be Stigmasterol as shown in figure 4:

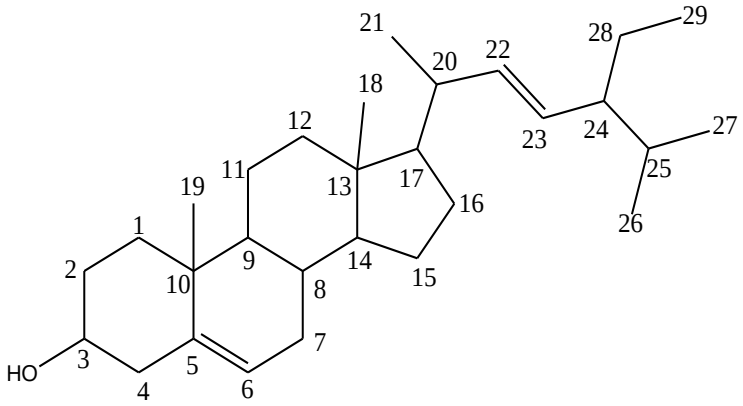

Figure 4: Stigmasterol

Table 1: Comparison of 1D NMR data of compound RO with literature

Carbon atom	**^{13}C NMR RO**	**^{13}CNMR [11]**	**^{1}HNMR RO**
C-1	37.27	37.25	1.87
C-2	31.69	31.65	1.55
C-3	71.82	71.80	3.55
C-4	42.23	42.21	2.32
C-5	140.76	141.01	…..
C-6	121.72	121.65	5.29
C-7	31.92	31.89	1.47
C-8	31.89	30.96	1.73
C-9	50.15	50.21	0.94
C-10	36.52	36.51	…..
C-11	24.31	24.30	1.49
C-12	39.79	39.76	1.14
C-13	42.33	40.52	……
C-14	56.78	56.76	0.91
C-15	23.07	23.03	1.04
C-16	29.90	28.94	1.25
C-17	56.07	56.04	1.06
C-18	12.06	12.06	0.72
C-19	19.83	19.41	1.03
C-20	39.79	39.67	2.26
C-21	21.09	21.07	1.98
C-22	138.31	138.34	5.17
C-23	129.29	129.32	5.05
C-24	51.25	51.24	1.55
C-25	36.07	36.15	0.94
C-26	19.41	18.96	0.87
C-27	18.99	18.76	0.72
C-28	21.09	25.42	1.20
C-29	11.87	11.96	0.82

CONCLUSION

The white powder that was successfully isolated from the hexane fraction of the methanol leaves extract of *Gossypium hirsutum* was found to be Stigmasterol on the basis of spectra data and comparison with literature. To the best of our knowledge, this is the first report of the isolation of Stigmasterol from the leaves of *Gossypium hirsutum.*

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