

Isolation and Characterization of Beta-Sitosterol from ethyl acetate extract of root bark of *Terminalia glaucescens*

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Abstract- The aim of this study is to identify and characterized the bioactive compounds from the root bark of the plant. Preliminary phytochemical screening of the root bark extract of *Terminalia glaucescens* revealed the presence of steroids, terpenoids, saponins, flavonoids, tannins and cardiac glycoside. The plant has wide folk medicinal use in traditional medicine. The air dried root bark was pulverized to powder, subjected to hot extraction (soxhlet) with methanol, and fractionated into n-hexane, ethyl acetate, and n-butanol fractions. Ethyl acetate as bioactive fraction based on sensitivity test was subjected to TLC and column chromatography. The isolated compound was colourless powder, which was further subjected to IR, UV, ¹³CNMR and ¹HNMR for proper characterization and elucidation of the structure. The compound was concluded as β-Sitosterol.

Index Terms- Terminalia glaucescens, Isolation and Characterization

I. INTRODUCTION

Medicinal plants have been in use for the eradication of human sufferings since ancient times. In light of their established therapeutic efficiency, the pharmaceutical industries started to use crude extracts of medicinal plants for manufacturing drugs (Ali, et al., 2000). The root bark of *Terminalia glaucescens* have been used in traditional medicine for treatment of dysentery, fever, diarrhea, wound, tooth decay, ulcers, typhoid fever and various stomach related problems.

Terminalia glaucescens is flowering plant tree (angiosperm) belong to family Combretaceae. It is commonly found in West Africa especially in Savannah regions. The plant is the most important medicinal species of the genus *Terminalia*. It is abundant in Nigeria. The plant is commonly called bausha (Hausa), Idi Odan (Yoruba), Edo (Igbo) while the local dilate where the plant was collected is called palma (Bura – Babur). *Terminalia glaucescens* is a tree up to 20 m high, bole usually short and gnarled. The bark is dark grey, deeply fissured; slash yellowish or reddish rapidly turning darker. Sometimes shoots and young foliage have densely hairy leaves, about 8.5-15 cm long, and 2.5-7.5 cm broad. Flowers are greenish-white, very small and strongly scented with brown hairs at the base of the style. Wood is pale yellow-brown, hard and coarse textured.

The purpose of this study is to identify and characterize the bioactive compound (s) from the root bark of *Terminalia*

glaucescens. In this paper, we report the isolation and characterization of known compounds from the plant namely β-sitosterol.

II. EXPERIMENTAL

Collection, Identification and preparation of plant materials.

The plant materials were collected from Hyera Road of Shaffa District, Hawul Local Government Area, Borno State of Nigeria. The plant was identified and the herbarium (voucher) specimen number UDUS/Bio/12/113 was prepared and deposited at the Herbarium of Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, by A. M. Umar (Taxonomist). The root bark of the plant was air dried under shed, then pulverized into powder with the aid of pestle and mortar. The powder obtained from the plant was then sieved and stored in polythene bags until required for use (African pharmacopoeia, 1985)

Extraction and Isolation

Six hundred gram (600g) of powdered root bark was extracted by soxhlet extractor used 1500ml of methanol as solvent at temperature of 85 °C, was concentrated using hot air sterilizing cabinet at 60°C and yield 123.11gram of methanol crude extract. Split method of separation was adopted according to (Abubakar, 2009). The n- hexane was directly added to crude methanol extract and was vigorous stirring before filtration and the filtrate are all n-hexane soluble portion, which is the n-hexane fraction while the residue was allowed to dry and same method was repeated with ethyl acetate, n- butanol and finally the residue obtained is methanol fraction. N-hexane, ethyl acetate, n - butanol, and methanol fractions were obtained and were concentrated at 60°C in hot air sterilizing cabinet. 100 ml burette was use as a Column with 50g of silica gel as a stationary phase while mobile phase was petroleum ether hundred percent followed by 9:1 ratio of petroleum ether and ethyl acetate as eluting solvent. The column was parked by wet parking method, after parking was allowed overnight with 3g of concentrated ethyl acetate fraction was dissolved in pet ether solution and soaked with cotton wool was placed on top of silica gel in the column. Between the cotton and the top of silica gel there was disc made of filter paper and the bottom of the column there was also another cotton wool. 2.4ml per minute each were collected in collection bottles range from 1 to 50. The column fraction's

profiles were monitored by TLC to confirming the similarities of elutes based on the number and color (s) of the spot (s).

Thin Layer Chromatography

Commercially pre-coated TLC silica gel plate was used a line was drawn with a pencil 2cm at the bottom from one end of the plate. The sample(s) were dissolved in little ethyl acetate solution and was spotted on the line drawn on the plate by capillary tube and then allowed to dry. The dry plates were placed into the chroma tank contained (9:1) ratio of chloroform and methanol, the tank was covered. The solvent rose up on the plate by capillary action, when the solvent front was just about 2cm to the upper end of the plate, the plate was removed and a line was drawn to mark the position of the solvent front. The plates were allowed to dry and the spots were developed by spread with 5% H₂SO₄ as spraying reagent. The R_f value of the spots were measured using meter rule.

Tests for steroid

Salkowski reaction: A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution. A reddish color was seen in the upper chloroform layer (Harbone, 1984).

Liebermann burchard reaction: A few crystals were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by addition of 2-3 drops of acetic anhydride. Solution turned violet blue and finally green (Harbone, 1984).

Spectroscopic characterization

The spectroscopic methods were used to elucidate the structure of isolated compound. Among the spectroscopic techniques IR, ¹H-NMR, ¹³C-NMR and UV were carried out. The infra red spectrum was recorded on FTIR -8400S, Shimadzu, Japan, from Central Lab. UDUS. ¹H-NMR and ¹³C-NMR spectra were recorded using CDCl₃ as solvent on Topspin (300MHz) Bruker, Germany, from University of Pretoria, South Africa. and UV spectra on UV/vis (SP3000plus) Kyoto, Japan from pharm. Chem. UDUS.

III. RESULTS AND DISCUSSION

The IR absorption spectrum showed absorption peaks at 3373.6cm⁻¹ (O-H stretching.); 2940.7 cm⁻¹ and 2867.9cm⁻¹ (aliphatic C-H stretching); 1641.6cm⁻¹ (C=C absorption peak); other absorption peaks includes 1457.3cm⁻¹ (CH₂); 1381.6cm⁻¹ (OH def), 1038.7cm⁻¹ (cycloalkane) and 881.6 cm⁻¹.

¹HNMR (CDCl₃, 300MHz) has given signals at δ 3.2(1H, m, H-3), 5.26 (1H, m, H-6), 5.19(1H, m, H-23), 4.68(1H, m, H-22), 3.638(1H, m, H-3), 2.38(1H, m, H-20), 1.8-2.0 (5H, m) ppm. Other peaks are observed at δ 0.76-0.89 (m, 9H), 0.91-1.05 (m, 5H), 1.35-1.42 (m, 4H), 0.69-0.73 (m, 3H), 1.8-2.00 (m, 5H), 1.07-1.13 (m, 3H), 1.35-1.6 (m, 9H) ppm.

¹³CNMR (CDCl₃, 100MHz) has given signal at 150.98, 145.2 (C-5), 139.8 (C-22), 121.7, 118.89(C-6), 79.03 (C-3), 55.3(C-14), 55.18(C-17), 50.45 (C-9), 48.3 (C-9), 40.8 (C-20), 40.1(C-12), 39.2 (C-13), 38.9 (C-4), 38.6 (C-12), 37.18 (C-1), 37.12 (C-10), 36.3(C-8), 35.59(C-20), 34.29 (C-22), 34.24

(C-7), 32.66 (C-8), 29.86 (C-25), 29.71 (C-16), 28.41 (C-2), 28.1 (C-15), 27.4 (C-28), 26.1 (C-11,26), 21.6 (C-27), 19.32 (C-19), 17.71 (C-21), 15.6 (C-18, 29).

Phytochemical analysis (Salkowski's test and Liebermann-Burchard test) of the compound confirm its steroidal nature. Since, the NMR machine indicated steroidal nucleus and the compound gives positive test for steroids so all of the other structures other than steroids were rejected. Based upon the functional group analysis it was found that the nature of oxygen was hydroxyl, also supported by IR spectroscopy (Shimadzu, IR). This implies presence of one double bond in the structure. So, the steroids with other functional groups were rejected. Also on considering the nature of oxygen as hydroxyl and presence of one double bond, the general formula for the compound is C_nH_{2n-6}, the exact molecular mass for the formula is found to be 414.37 and the chemical formula could be tentatively C₂₉H₅₀O. Therefore it must be a tetra cyclic compound. Based on the melting point and other related data (IR, NMR, UV and MS) the structure of the isolated compound is proposed as

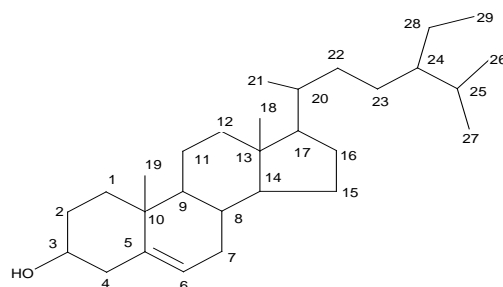


Fig. 1 β-sitosterol (Stigmast-5-en-3β-ol)

The compound is a colourless crystalline compound, λ_{max} in CHCl₃: 220 nm. On subjecting to IR spectroscopic analysis, absorption bands appeared at 3570.36 – 3186.51 cm⁻¹ that is characteristic of O-H stretching, 2864.39 cm⁻¹ is due to aliphatics or C-H stretching or (CH₃), 1585.54 cm⁻¹ due to double (C=C) stretching, 1016.52cm⁻¹ due to (C-O). Other absorption frequencies include 3838.47cm⁻¹ due to combination of absorption and 2353.23cm⁻¹ due to overtone of the absorption, at 1269.2 cm⁻¹ is a bending frequency for cyclic (CH₂)_n. The absorption frequency at 783.13 cm⁻¹ signifies cycloalkane. The out of plane C-H vibration of unsaturated part was observed at 609.53cm⁻¹. These absorption frequencies resemble the absorption frequencies observed for β-sitosterol as resembled data published by (Arjun *et al*, 2010).

The ¹HNMR spectrum (300MHz, CDCl₃) of compound fig. 1 has revealed a one proton multiplet at δ 2.41, the position and multiplicity of which was indicative of 3H of the steroidal nucleus. The typical 6H of the steroidal skeleton was evident as a multiplet at δ 5.39 that integrated for one proton. The spectrum further revealed signals at δ 1.47 and δ 1.19 (3H each) assignable to two tertiary methyl group at C- 18 and C-19 respectively. The ¹HNMR spectrum showed two doublets centered at δ 0.90 (J = 6.7Hz) and δ 0.89 (J = 6.7Hz) which could be attributed to two methyl groups at C-26 and C -27 respectively. The doublet at δ 1.62 (J = 6.5Hz) was demonstrative of a methyl group at C-21. On the other hand, the triplet of three proton intensity at δ 0.88 could be assigned to the primary methyl group at C- 29. This

compound is having six methyl, eleven methylene and three quaternary carbons with a hydroxyl group. The above spectral features are in closed agreement to those observed for β – Sitosterol according to (Manoharan et al., 2005 and Escudero *et al.*, 1985).

The $^{13}\text{C-NMR}$ has shown recognizable signals 179.21 and 129.69 ppm, which are assigned C5 and C6 double bonds respectively. The value at 24.67 ppm corresponds to angular carbon atom (C19). Spectra show twenty nine carbon signal including six methyls, nine methylenes, eleven methane and three quaternary carbons. The alkene carbons appeared at δ 179.21 and 129.69. The structure was simulated using ACD/NMR program to obtain the chemical shifts of both proton and carbon. On comparison the standard data matched with the simulated data which supports the proposed structure of this compound as β – Sitosterol.

IV. CONCLUSION

Stigmast-5-en-3 β -ol (β -Sitosterol) was isolated and characterized from ethyl acetate extract of root bark of *T. glaucescens*. β -Sitosterol reduce carcinogen-induced cancer of the colon. It shows anti-inflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin releasing and oestrogenic effects and inhibition of spermatogenesis. Beta-sitosterol is mainly known and used for its cholesterol lowering property. But studies have shown that the phytochemical may have other health benefits: easing symptoms of benign prostatic enlargement, reducing risk of cancer and prevention of oxidative damage through its antioxidant activity.

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