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Isolation of Stigmasterol from the Root Bark of *Annona senegalensis Pers.* (Annonaceae)

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ABSTRACT

Annona senegalensis (Pers.), commonly known as wild custard apple belongs to the family Annonaceae. The root bark of the plant was air dried, pulverized and extracted with methanol. The extract was then subjected to general phytochemical screening and chromatographic analysis. General phytochemical screening of the extract showed the presence of tannins, saponins, alkaloid, triterpenes, steroids, flavonoids and cardiac glycosides. The column chromatographic analysis of the chloroform fraction of the crude extract led to the isolation of a white crystalline powder, coded JR. The structure of JR was elucidated using IR, 1D and 2D NMR analysis. The compound was identified as stigmasterol. Despite the reports on isolation of essential oils from this plant as well as its ethnomedicinal uses, there was scanty literature on the isolation of stigmasterol from the root bark of the plant, hence this study.

Keywords: *Annona*, *senegalensis*, stigmasterol, IR, NMR

INTRODUCTION

Medicinal plants are plants that possess therapeutic properties or exert beneficial pharmacological effects on human body [1]. These plants grow naturally and accumulate secondary metabolites known as natural products such as alkaloids, steroids, terpenes, flavonoids, saponins and tannins. These secondary metabolites may act individually, additively or synergistically to improve health [2].

Most traditional medicine practitioners rely on plants which contain some of these bioactive secondary metabolites. Natural products, particularly those derived from plants have been used by man to sustain his health since emergence of medicine. Over the past century, the phytochemicals in plants have been a pivotal pipeline for pharmaceutical discovery [3]. Natural products include any substance produced by living organisms [4]. Plants have a long history of

therapy all over the world and still make an essential part of traditional medicine. Interest in medicinal plants as a reemerging health aid have increased as a result of rising cost of prescription drugs in maintenance of personal health and wellbeing and the bioprospecting of new plant derived drugs. The main benefit of using plant derived medicines is that, they are relatively safer than synthetic alternatives thus offering profound therapeutic benefits at affordable cost [5].

Annona senegalensis popularly known as wild custard apple (English), Dukuu hi (Fulani), Gwandardaji (Hausa), Abo (Yoruba), Uburuocha (Igbo) is one of such medicinal plants used in ethnomedicinal treatment of diseases in Nigeria. It is a shrub or small tree 2-6 m tall, but may reach 11 m under favourable conditions. The bark is smooth to rough, silver grey or grey-brown. Leaves are alternate, simple, oblong, ovate or elliptic, green to bluish green, almost without hair on upper surface but often with brownish hair on the lower surface. Flowers are up to 3 cm in diameter on stalks 2 cm long, solitary or in groups of 2-4, arising above the leaf axils [6]. It grows wild in tropical Africa.

Wild fruit trees of this species are found in semi-arid to sub-humid regions of Africa. The species occur along river banks, fallow land, swamp, forests and at the coast. It commonly grows as a single plant in the understorey of savannah woodlands [7]. It is very common in Northern Nigeria, primarily in Nasarawa, Kaduna, Kano, Plateau, and Niger States and in the Federal Capital Territory, Abuja [8]. Numerous ethnomedicinal uses have been attributed to the different parts of the plant and it is highly reputable for its great medicinal value. All parts of *A. senegalensis* plant have been found useful for traditional medicine applications. The plant is commonly used in Nigerian folk medicine in the treatment of malaria [9]. The root is used in conditions such as difficulty in swallowing, gastritis, snake bites, male sexual impotence, erectile dysfunction, tuberculosis and as antidote for necrotizing toxins [6]. The root bark is effective in infectious diseases [10].

Despite the reports on isolation of essential oils from the root bark of this plant as well as its ethnomedicinal uses, there is scanty literature on the isolation of stigmasterol from the root bark of the plant. The present study is therefore aimed to extract, isolate and characterize by spectroscopy the stigmasterol from the root bark of *Annona senegalensis.*

MATERIAL AND METHODS

Plant Collection, Identification and Preparation

The plant sample of *Annona senegalensis* was collected at Bida Local Government Area of Niger state, Nigeria. It was identified and authenticated by Mallam NamadiSunusi of Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, by comparing with herbarium reference. Voucher specimen number 0190 was obtained. The root bark was air dried, pulverized, labelled and stored at room temperature for use.

Extraction and Partitioning

The root bark of the plant (900 g) was extracted with methanol using cold maceration for 72 hours. The extract was evaporated *in-vacuo* in rotary evaporator to yield a brownish residue (90 g) labelled as crude methanol extract (CME). The crude extract was then suspended in distilled water and filtered to obtain water soluble and water insoluble portions. Both portions were successively partitioned with n-hexane, chloroform and ethyl acetate to afford hexane fraction (HF), chloroform fraction (CF) and ethyl acetate fraction (EF) 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 [11].

Chromatographic Separation

The chloroform fraction (8 g) was subjected to column chromatography using silica gel (60-120 mesh) as the stationary phase. The fraction was dissolved using ethyl aetate and then mixed with silica gel to form slurry which was allowed to dry. It was then loaded on the already packed column and was gradiently eluted starting with 100% hexane followed by hexane : ethylacetate 98:2 and increasing the polarity gradually by 2% up to 100% ethylacetae, then finally eluted with methanol. A total of 63 collections of 100ml each were made and were subsequently combined based on their TLC profiles to give 21 pooled fractions coded A-T. Collections 38-47 coded O were pooled together on the basis of their TLC profile and was further subjected to column chromatography. The fraction was eluted isocratically on a silica gel packed column with DCM:ethylacetae 6:4. A total of 95 collections were made and combined into 5 fractions coded P_A, P_B, P_c, P_d and P_F. P_F was further washed with methanol to produce a white amorphous powder

coded JR. JR was a white powdered substance with a melting point of 135-137 ºC, gave a single spot when subjected to TLC using 100% EA and H:EA 1:1 as solvent system with Rf values of 0.4 and 0.3 respectively. JR was further subjected to physicochemical test and spectral analysis using IR and NMR to ascertain the chemical structure.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the methanol root bark extract of *Annona senegalensis* (Pers.) revealed the presence of alkaloids, flavonoids, terpenoids/steroids, tannins, carbohydrate, saponin and cardiac glycosides

The IR spectrum of JR showed characteristic absorption frequencies 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 aromatic C=C in plane stretching vibrations and 1449.9 cm^{-1} due to bending frequency of cyclic methylene and 1028 cm^{-1} is due to cycloalkane [12].

The H NMR ($CDCl_3$) spectrum of JR varied between 0.71 and 5.38 revealed the presence of six methyl protons at δ0.71 (C18), 1.04 (19), 0.83 (21), 0.86 (26), 0.82 (27) and 0.88 (29). This proton signals between 0.7- 1.8 are attributed to resonance of overlapping methyl and methylene protons, a characteristic framework of steroids [13,14]. The carbinol proton (H-3) resonates as a multiplet at 3.54 ppm. It also revealed the presence of olefinic protons signal at δ5.38(m), 5.18 (m) and 5.04 (m).

The ^{13}C NMR ($CDCl_3$, 400MH_Z) showed the presence of 29 carbon atoms comprising of six methyl carbons, nine methylene carbons, eleven methine carbons and three quaternary carbons. These are all characteristic resonances of a sterol with two olefins and an alcohol [15]. It showed some recognizable signals at δ140.84 and 121.72 for C5 and C6 double bond respectively and δ138.26 and 129.42 for C22 and C23 double bond respectively as well as signals at δ11.87 and 19.41 for the angular methyl carbons (C18 and C19) respectively. The deshielded signal at δ71.8 was due to C3 with hydroxyl group attached to it [14]. All these resonances are similar to that of stigmasterol reported by Pateh *et al*. [16]

The results ^1H-^1H COSY correlation confirmed the relationship between the various protons in the molecule. The ^1H-^1H COSY experiment established the correlations between the protons at H3(5.35) # H2(1.53), H3(5.35) # H4(2.30), H22(5.18) # H23(5.05), H25(1.68) # H26(0.86).

Based on the comparison of the IR and NMR data with literature data, compound JR was suggested to be stigmasterol as shown in figure 3.

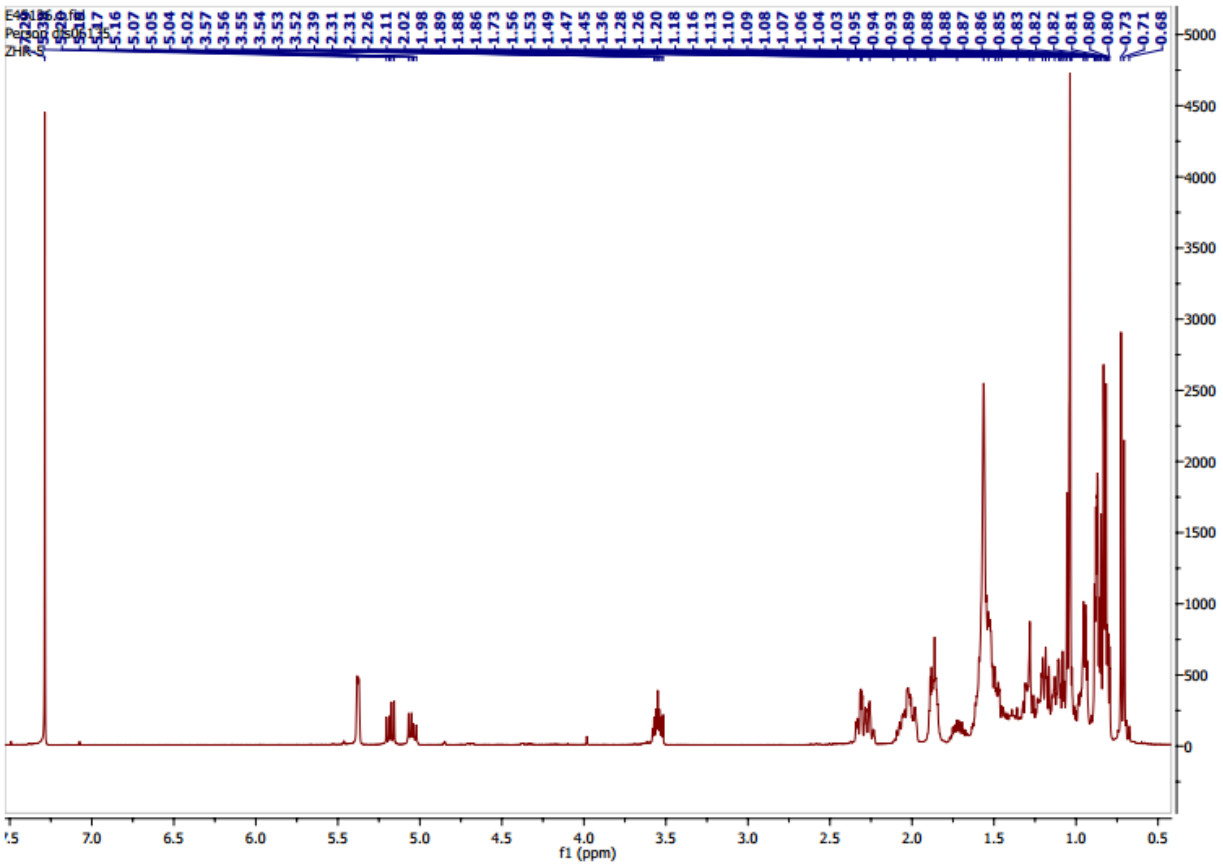

Figure 1: 1H-NMR Spectrum of compound JR

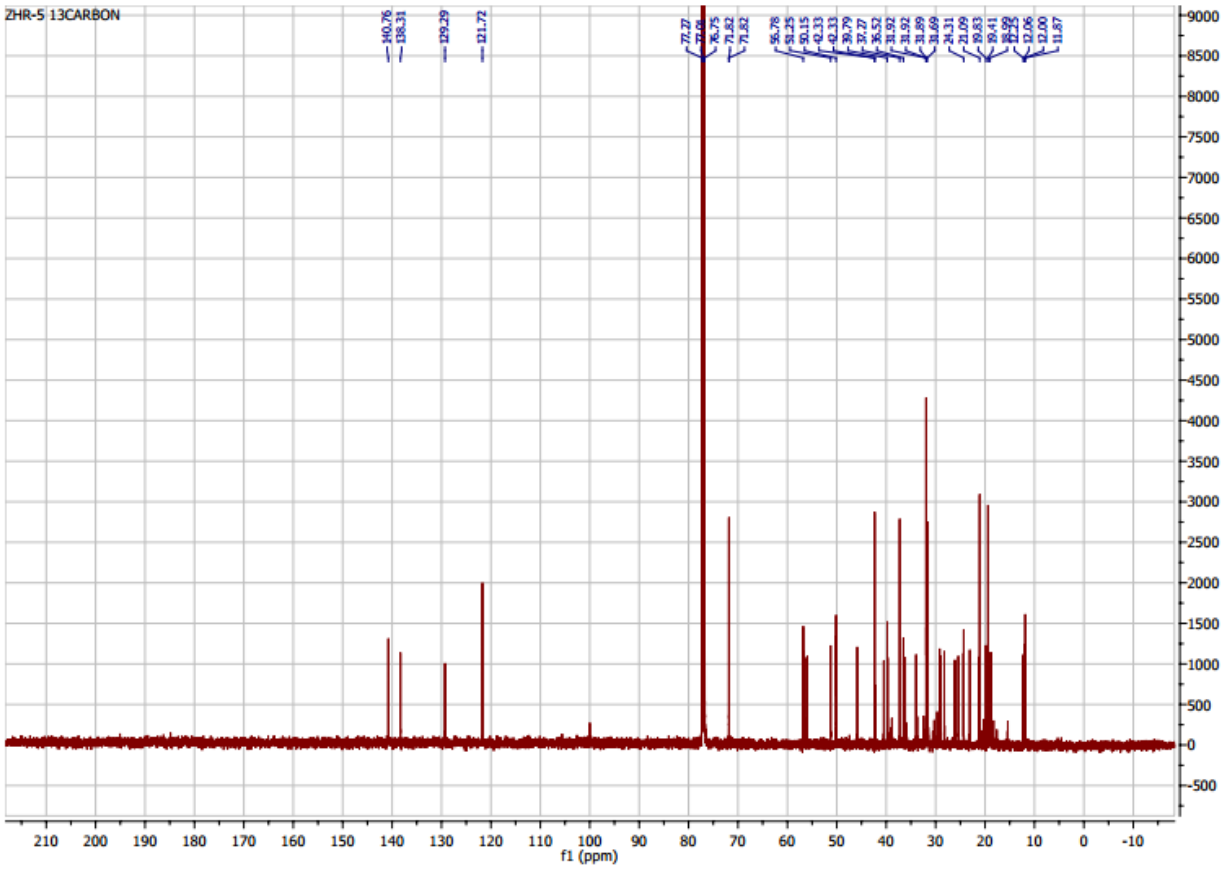

Figure 2: ^{13}C-NMR spectrum of compound JR

Table 1: Comparison of 1D and 2D NMR data of compound JR with literature [16]

Pos. of Carbon	13C NMR (JR)	13C NMR [16]	1H NMR (JR)
1	37.27	37.30	1.08
2	31.69	31.60	1.53
3	71.82	71.80	3.53
4	40.49	42.20	2.30
5	140.84	140.80	---
6	121.72	121.70	5.38
7	31.89	31.90	1.47
8	31.92	31.90	1.86
9	50.15	51.20	0.95
10	36.52	36.50	---
11	21.22	21.10	1.56
12	39.89	39.80	2.03
13	42.33	42.30	---
14	56.82	56.80	1.02
15	24.34	24.30	1.10
16	28.59	28.30	1.74
17	56.02	56.00	1.14
18	11.87	11.00	0.71
19	19.41	21.20	1.04
20	36.17	36.20	1.38
21	19.06	18.80	0.83
22	138.26	138.30	5.18
23	129.42	129.30	5.05
24	45.86	51.20	0.96
25	29.18	31.90	1.68
26	19.83	21.20	0.86
27	18.99	19.00	0.82
28	23.09	25.40	1.28
29	12.25	12.10	0.88

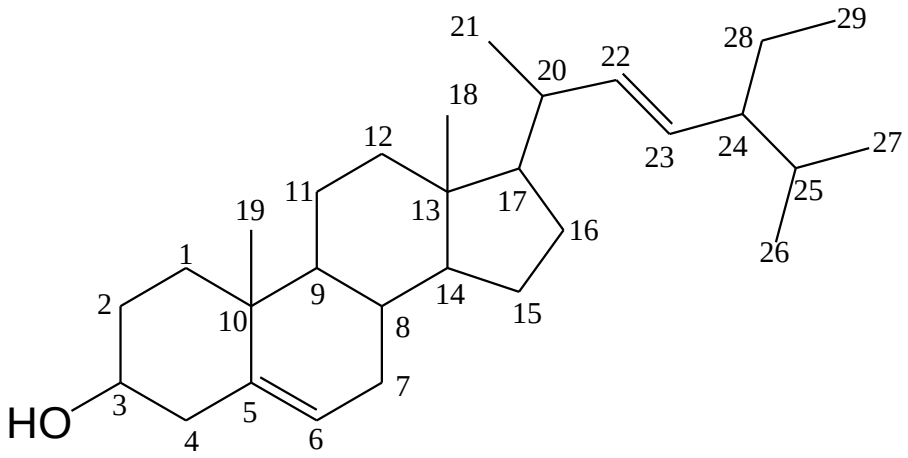

Figure 3: Structure of JR (Stigmasterol)

CONCLUSION

Chromatographic separation of the crude methanol extract of *Annona senegalensis* led to the isolation stigmasterol. IR, 1D and 2D NMR were used in the elucidation of the chemical structure of the isolated compound.

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