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Isolation and Characterisation of Stigmasterol and β–Sitosterol from *Anthocleista djalonensis* A. Chev.

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Anthocleista djalonensis* A. Chev. is a plant with several chemical constituents which accounts for its ethno-pharmacological uses**.** The present study is aimed at identifying and characterizing the active principles from the roots of the plant.
Place and Duration of Study: The study was carried out at the Department of Organic Chemistry, Rhodes University, Grahamstown, South Africa between March and July 2016.
Methodology: The root powder was subjected to maceration with methanol to obtain the crude extract. The methanol extract was fractionated using hexane, ethyl acetate and acetone successively. The acetone extract was thereafter subjected to column chromatography to isolate any pure components.
Results: White needle-like crystals were obtained which on spectral analysis(IR, ^{1}H NMR, ^{13}C NMR, 2D- NMR, and ESI-MS) were identified as a mixture of stigmasterol and β-sitosterol.
Conclusion: The compounds isolated were identified as stigmasterol and β-sitosterol.

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Keywords: Anthocleista djalonensis; chromatography; maceration; stigmasterol; β- sitosterol.

1. INTRODUCTION

Anthocleista djalonensis A. Chev.(Loganiaceae) is a medium-sized tree (30 – 45 m tall) of West tropical Africa [1]. The root bark of the plant is reported to contain irlbacholine [2], which showed very significant antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigatus* and *Trichophyton rubrum*. Other compounds isolated from the plant include triterpenes, a monoterpene-diol djalonenol, a dibenzo-pyrone djalonensone, an iridoid glycosidesweroside (djalonenoside) [3] as well as amplexine and axanthone lichexanthone [4]. The stem bark is also reported to contain the phthalide djalonensin [4]. The stem, roots and leaves of *A. djalonensis* are used traditionally to treat malaria, jaundice, diabetes and abscesses [1,5]. The Igbos in Nigeria use the seeds, barks and roots as antipyretic, a laxative and remedy for various stomach disorders [6]. It is used to treat epilepsy in Ghana [5,7] and in Southern Nigeria, the leaves and stem bark are used as a remedy for malaria [7,4,8]. It is also reported that the plant is useful as febrifuge, abortifacient and relief for pains. The roots of the plant have been reported to contain steroids [9,10] and the present study reports on the isolation of two steroids from the roots of the plant.

2. MATERIALS AND METHODS

2.1 Plant Material

The roots of *Anthocleista djalonensis* were collected from trees growing freely in ZakiBiam, Benue State Nigeria. The plant was identified by Mr Ibe Ndukwe, a taxonomist in the Forestry Department, Michael Okpara University of Agriculture Umudike Nigeria and voucher specimens No: AD/124 had been deposited at the Forestry Department, Herbarium of the University. The roots were dried under a shade for three weeks and milled at the Chemistry Department, University of Agriculture Makurdi using a Thomas model 4 Willey Mill.

2.2 Extraction of Plant Material

The extraction as described by Okoro et al. [11] was adopted. The extracts were concentrated to give a hexane, ethyl acetate and acetone extracts.

2.3 Isolation and Purification of Compounds

The acetone extract being the most active (biological activity of this extract will be published elsewhere) against tested biological assays was subjected to column chromatography on silica gel (Merck 70-30 mesh, bed surface area 500 m^2/g pore volume 0.75 cm^2). The column was eluted gradient wise with hexane and dichloromethane and the eluates collected in 50 mL fractions. The fractions were analysed by TLC and similar fractions combined to obtain 23 fractions which includes fraction AD-6.

2.4 Purification of Fraction AD-6

Fraction AD-6 (11.715 mg) was discovered to have crystals. The crystals were washed separately and repeatedly with Hexane (50 mL). TLC with hexane and dichloromethane (1:2) gave a single spot with Rf 0.62. The purified fraction was labelled AD-03.

2.5 Test for Steroid

2.5.1 Salkowski reaction

A few crystals of AD-03 were dissolved in chloroform and a few drops of concentrated sulfuric acid were added to the solution, A reddish color in the upper chloroform layer [12] was observed by AD-03 indicating presence of steroids.

2.5.2 Liebermann-burchard reaction

A few crystals of AD-03 were dissolved in chloroform and few drops of acetic anhydride and concentrated sulfuric acid were added to the chloroform solution. Violet blue and finally green color [12] was formed by AD-03 indicating the presence of steroids.

2.6 Spectroscopic Characterization

Spectroscopic methods were used to elucidate the structure of the isolated compound. The spectra (^1H, ^{13}C, 2D NMR were recorded using $CDCl_3$ as solvent on a Bruker Avance II 600 NMR spectrophotometer. ESI were recorded on a high resolution Waters API Q-TOF Ultima ESI mass spectrometer, from Stellenbosch University, South Africa

3. RESULTS

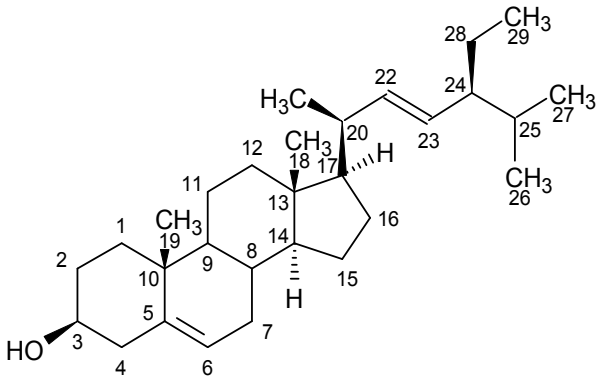
The melting point of AD-03 was 145-147ºC. The mass spectrum of AD-03 showed a molecular ion at 415.2117

4. DISCUSSION

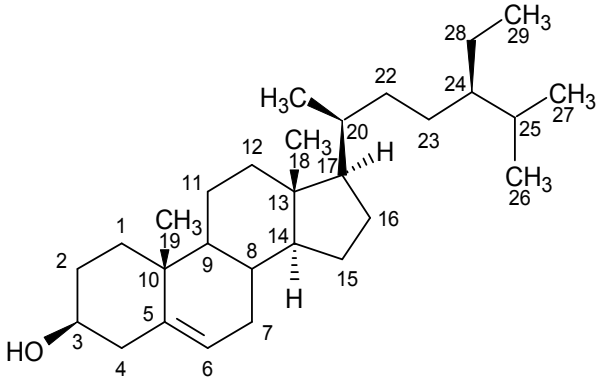
The result of the positive tests for steroids by AD-03 showed the compound containing a steroidal skeleton with a melting point of 145^{o}C.The ^{1}H NMR data of AD-03 showed 3 olefinic protons at δ 5.16, δ 5.33 and δ 5.36(H-22, 23 and 6). A triplet of a doublet of doublet was observed at δ 3.54(H-3). While carbon 18,19, 21. 26. 27, and 29 revealed the presence of six methyl protons with their signals at δ 1.27, 0.71,1.17, 0.86, 0.94, 1. 04. These signals and assignments is in accordance with reported values [13-17]. The 13C NMR showed three quaternary carbons, eleven methane, nine methylenes, and six methyl carbons [15,18]. C5 and C6 alkene carbons with double bonds revealing distinct signals at 141.01 and 121.65 [19,20]. Angular carbon atom signal(C-19, C-18) was also recognized at 19.41 and 12.06 [13]. A signal at 71.80 (C-3) indicated a hydroxyl group [13,14]. The COSY, HMQC and HMBC correlations aided in the assignments of values of all protons for 1H NMR and 13C NMR.

Table 1. ^{13}C NMR and ^{1}H NMR spectral data for AD-03 recorded in $CDCl_3$ (600 MHz)

Carbon atoms	^{13}C NMR experiment	^{13}C NMR literature	^{1}H NMR experiment	^{1}H NMR literature	Type of carbon
C-1	37.25	36.72			CH_2
C-2	31.65	29.71			CH_2
C-3	71.80	71.97	3.54(tdd,1H)	3.53(m,1H)	CH
C-4	42.21	42.35			CH_2
C-5	141.01	140.94			C=C
C-6	121.65	121.32	5.36(m,1H)	5.38(s,1H)	C=CH
C-7	31.89	31.71			CH_2
C-8	30.96	29.24			CH
C-9	50.12	50.03			CH
C-10	36.51	36.16			C
C-11	24.30	24.32			CH_2
C-12	39.76	39.82			CH_2
C-13	40.52	40.45			C
C-14	56.76	56.90			CH
C-15	23.06	24.32			CH_2
C-16	28.94	28.90			CH_2
C-17	56.04	56.03			CH
C-18	12.06	12.06	1.27(d,3H)	1.29(d,3H)	CH_3
C-19	19.41	19.06	0.71(d,3H)	0.74(d,3H)	CH_3
C-20	39.67	39.82			CH
C-21	21.07	23.12	1.17(d,3H)	1.20(d,3H)	CH_3
C-22	138.34	138.40	5.16(m,1H)	5.07(m.1H)	C=C
C-23	129.32	129.34	5.33(m,1H)	5.20(m,1H)	C=C
C-24	51.24	51.26			CH
C-25	36.15	34.01			CH
C-26	18.96	21.12	0.86(d,3H)	0.84(d,3H)	CH_3
C-27	18.76	22.82	0.94(d,3H)	0.97(d,3H)	CH_3
C-28	25.42	25.32			CH_2
C-29	11.96	12.06	1.04(t,3H)	1.04(t,3H)	CH_3



Stigmasterol



β-Sitosterol

Fig. 1. Structures of stigmasterol and β-sitosterol

5. CONCLUSION

The compound isolated from Anthocleista djalonensis acetone was a mixture of Stigmasterol and β- Sitosterol. The structure identification employed physical and spectroscopic methods. The characterization was made possible comparing the physical properties with that found in literature. Further studies on the pharmacological activities should be undertaken.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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