ISOLATION AND CHARACTERIZATION OF STIGMASTEROL AND B-SITOSTEROL FROM ETHYL ACETATE EXTRACT OF *Adenodolichos paniculatus* (FABACEAE)

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Abstract: The leaves of *Adenodolichos paniculatus* (Fabaceae) was collected, identified, dried and pulverized. The pulverized plant material was subjected to microwave assisted extraction using n-hexane, chloroform, ethyl acetate and methanol. Column chromatography of the ethyl acetate extract led to a number of fractions. TLC fingerprinting and the spraying reagent (10% H_2SO_4) was used to identify the fraction containing phytosterols. The isolation and purification afforded a white crystalline powder which was subjected to physical (melting point determination), and spectroscopic identification using IR, ^1H and ^{13}C NMR. The compound was identified as a mixture of stigmasterol and β-sitosterol.

Keywords: Stigmasterol, β-Sitosterol *Adenodolichos paniculatus*, Fabaceae

Introduction

The increase in drug resistance recorded by pathogens is growing into a worldwide problem. As a result, there is an urgent need to develop new antimicrobial drugs with better activity in order to overcome microbial drug resistance (Folayan *et al.*, 2008).

Medicinal plants represent the main source of pharmaceuticals and health care products (Ahmed *et al.*, 2006). Medicinal plants contain some compounds which have definite physiological actions on animals and humans. These substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Doge *et al.*, 2005). The antibacterial activities of stigmasterol and beta sitosterol have been reported in many literatures (Amit *et al*., 2012; Sileshi *et al.*, 2012; Soodabeh *et al.*, 2014).

Research has indicated that stigmasterol may be useful in prevention of certain cancers, including ovarian, prostate, breast, and colon cancers (Soodabeh *et al.*, 2014). It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties (Panda *et al.*, 2009). Stigmasterol and sitosterol are two phytosterols well spread in plants and animals as well as fungi, and have structural similarity to cholesterol.

The most important benefit for these two secondary metabolites is their enrolment amongst the health promoting constituents of natural foods which contains them (Karan *et al.*, 2012). Microwave- assisted extraction (MAE) is a quite new method used for the extraction of natural products (Afoakwah *et al.*, 2012). Ganzler *et al.* (1990) showed that Microwave-assisted extraction for biologically active compounds has so many advantages over other conventional extraction methods. Microwave-assisted extraction method required shorter time, less solvent, provided higher extraction rates and lower costs. Numerous Biologically active compounds have been extracted by application of microwave-assisted extraction (Monica *et al.*, 2010).

Materials and Methods

The leaves of *Adenodolichos paniculatus* were collected from Makurdi, Benue State, Nigeria. The plant was identified and authenticated at the Herbarium unit, Department of Biological Sciences, Ahmadu Bello University, Zaria and a specimen voucher number 3107 was deposited. The leaves were air dried, powdered, and stored in air tight containers for laboratory analyses.

Extraction and isolation

1 kg of the dried pulverized leaf of *Adenodolichos paniculatus* was extracted exhaustively using a commercial microwave, using n-hexane, chloroform, ethyl acetate and methanol respectively to get four extracts upon evaporation of the solvent at reduced pressure using a rotary evaporator. The ethyl acetate extract (5 g) was chromatographed on a silica gel column and eluted using solvent mixtures of n-hexane/ethyl acetate 95:05, 90:10, 85:15 and 80:20, respectively to yield 25 fractions. Compound X1 (5 mg) was obtained from fraction 5 (eluate of n-hexane/ethyl acetate = 80:20) and upon preparative thin layer chromatography using a solvent system of n-hexane/ethyl acetate 75:25.

Equipment

Silica gel 60 F_{254} (Merck) was used for TLC analysis, column chromatography was performed using Merck silica gel (60–120) mesh while spots on TLC plates were visualized by spraying with 10% H_2SO_4 followed by heating at 100°C for 5 min. The IR spectrum was measured on a Shimadzu FT-IR8 400S Fourier transform infrared spectrophotometer. Nuclear magnetic resonance (NMR)-spectra were recorded on a Bruker Avance spectrometer (400 MHz) for ^1H- and (100 MHz) for ^{13}C-NMR, internal standard was residual solvent signal with chloroform as a solvent.

Results and Discussion

Table 1 depicts the summary of ^{13}C and ^1H NMR spectral data for compound X1; while Table 2 displays comparison of 1D NMR Data of X1 with literature data (Luhata *et al.*, 2015).

The FTIR spectrum showed absorptions band for OH at 3306.1 cm^{-1}, CH_3 at 2944.6 cm^{-1} H_2 at 2832.8 cm^{-1}, unconjugated olefinic (C=C) at 2023.9 cm^{-1}, and also the bands observed in the finger print region are due to various C-H stretching and bending modes (Ododo *et al.*, 2016; Luhata and Mofiz 2015; Wade, 2006).

1H NMR spectrum showed the presence of fifty hydrogens: six methyl protons (CH_3) at δ 0.70, 0.96, 0.96, 0.96, 1.06 and 1.06, eleven methylene protons (CH_2) at δ 1.25, 1.25, 1.29, 1.38, 1.49, 1.52, 1.57, 1.60, 1.60, 2.04 and 2.23, nine methin protons (CH) at δ 1.25, 1.40, 1.44, 1.45, 1.47, 1.47, 1.64, 3.25 and 5.37 and one OH group the singlets at δ 0.70 and 1.03 confirming the presence of two (CH_3) attached to quaternary carbons. The appearance of the complex multiplet at δ 2.29 and 2.32 revealed the two (CH_2) adjacent to carbon attached to OH group. While the signal at δ 5.37 is due to methine proton on olefinic carbon (C6). However, lack of signal at C5, C10 and C13 shows lack of protons on these carbons, they are

quaternary carbons The signal at δ1.24 to 1.57 show an indication of methylene proton The methyl signals observed at δ 0.96, 1.24 and 1.26 is atypical of terminal methyl (CH_3) (Ododo *et al.*, 2016, Luhata *et al.*, 2015, Isah *et al.*, 2014).

Table 1: Summary of ^{13}C and ^{1}H NMR spectral data for compound X1

Position	^{13}C NMR	^{1}H, *J* (Hz)	Nature of Carbon
C-1	37.20		CH_2
C-2	29.70		CH_2
C-3	71.82	3.51 (m, 1H)	CH
C-4	42.22		CH_2
C-5	140.90		C
C-6	121.75	5.34 (s, 1H)	CH
C-7	31.85		CH_2
C-8	29.24		CH
C-9	50.05		CH
C-10	36.13		C
C-11	24.28		CH_2
C-12	39.72		CH_2
C-13	42.22		C
C-14	56.72		CH
C-15	24.67		CH_2
C-16	28.93		CH_2
C-17	55.97		CH
C-18	11.96	1.03 (d, 3H)	CH_3
C-19	19.39	0.71 (d, 3H)	CH_3
C-20	39.72		CH
C-21	22.99	0.91 (d, 3H)	CH_3
C-22	132.5		CH
C-23	124.44	5.14 (m, 1H)	CH
C-24	49.80		CH
C-25	33.88		CH
C-26	21.05	0.80 (d, 3H)	CH_3
C-27	22.70	0.82 (d, 3H)	CH_3
C-28	25.93		CH_2
C-29	11.84	0.83 (t, 3H)	CH_3

The 13C – NMR spectra data of compound X1 (δ 1H and δ 13C) agrees with those reported by Ododo*et al.* (2016), Luhata *et al.* (2015). The 13C-NMR has shown recognizable signals at 140.9 and 121.755 ppm which are assigned C5 and C6 double bonds respectively. The value at 19.397 ppm corresponds to angular carbon (C19) 132.50 ppm for C-22 and 124.448 ppm for C-23. Spectra show twenty-nine carbon signal including six methyls, nine methylenes, eleven methane and three quaternary carbons. The alkene carbons appeared at 140.9, 121.755, 132.50 and 124.448 ppm. (Prakash *et al.*, 2012; Luhata *et al.*, 2015) According to Prakash *et al.* (2012); Bulama *et al*, (2015); Luhata and Munkombwe (2015); Mofiz *et al.* (2015); Ododo *et al.* (2016), β-sitosterol and Stigmasterol have the same R_f value of 0.55 and are always in a mixture form. It is very difficult to obtain Stigmasterol in pure state. The only difference between the two compounds is the presence of C=C double bond at C22 and C23 in Stigmasterol and C-C single bond at C22-C23 in β-sitosterol. Furthermore, literatures have shown that sitosterol is difficult to be obtained in pure state (Luhata *et al.*, 2015). Therefore, compound (X1) is a mixture of β-sitosterol and Stigmasterol. β-sitosterol is colourless solid with a melting point of 147-149℃.

Table 2: Comparison of 1D NMR Data of X1 with literature data (Luhata *et al.*, 2015)

Carbon Atom	^{13}C NMR Experimental	^{13}C NMR Literature	^{1}H NMR Experimental	^{1}H NMR Literature	Type of Carbon
C-1	37.20	36.72			CH_2
C-2	29.70	29.71			CH_2
C-3	71.82	71.97	3.51 (m, 1H)	3.53 (m, 1H)	CH
C-4	42.22	42.35			CH_2
C-5	140.90	140.94			C
C-6	121.75	121.32	5.34 (s, 1H)	5.38 (s, 1H)	CH
C-7	31.85	31.71			CH_2
C-8	29.24	29.24			CH
C-9	50.05	50.03			CH
C-10	36.13	36.16			C
C-11	24.28	24.32			CH_2
C-12	39.72	39.82			CH_2
C-13	42.22	42.45			C
C-14	56.72	56.90			CH
C-15	24.67	24.32			CH_2
C-16	28.93	28.90			CH_2
C-17	55.97	56.03			CH
C-18	11.96	12.06	1.03 (d, 3H)	1.29(d, 3H)	CH_3
C-19	19.39	19.06	0.71 (d, 3H)	0.74(d, 3H)	CH_3
C-20	39.72	39.82			CH
C-21	22.99	23.12	0.91 (d, 3H)	1.20(d, 3H)	CH_3
C-22	132.5	138.40			CH
C-23	124.44	129.34	5.14 (m, 1H)	5.20(m, 1H)	CH
C-24	49.80	51.26			CH
C-25	33.88	34.01			CH
C-26	21.05	21.12	0.80 (d, 3H)	0.84(d, 3H)	CH_3
C-27	22.70	22.82	0.82 (d, 3H)	0.97(d, 3H)	CH_3
C-28	25.93	25.32			CH_2
C-29	11.84	12.06	0.83 (t, 3H)	1.04(t, 3H)	CH_3

Compound X: A mixture of β-sitosterol and stigmasterol

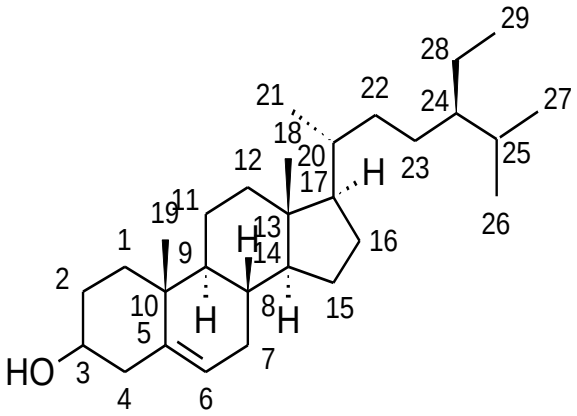

Fig. 1A: *β*-sitosterol

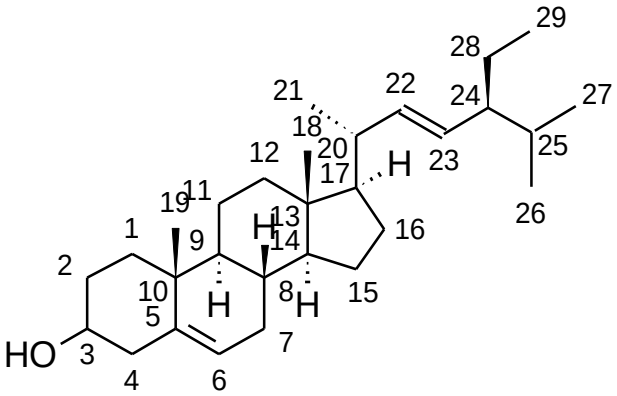

Fig. 1B: stigmasterol

According to the results above, compound (X1) isolated from the leaves extract of *adenodolichos paniculatus* was determined to be a mixture of stigmasterol and β-sitosterol (Fig. 1a & b). Well known phytosterols. The structure of the isolated compounds was identified on the basis of spectroscopic methods and by comparing with data reported in the literatures.

Conclusion

Stigmasterol and β-sitosterol are two phytosterols well spread in plants. The economic importance of these two secondary metabolites is their enrolment in the health promoting constituents of natural foods which contain them (Mofiz *et al.,* 2015). They both showed anti-inflammatory, anti-pyretic, antiarthritic, anti-ulcer, insulin-releasing and oestrogenic effects and inhibition of spermatogenesis (Bulama *et al.,* 2015) stigmasterol and Beta-sitosterol are mainly known and used for their, cholesterol lowering property, reducing the risk of heart disease (Luhata *et al.,* 2015), easing symptoms of benign prostatic enlargement, reducing risk of cancer and prevention of oxidative damage through its antioxidant activity (Prakash *et al.,* 2012). This shows that the use of *Adenodolichos paniculatus* by traditional healers especially in the treatment of heart diseases has been justified.

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