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ISOLATION OF *β*-SITOSTEROL AND LUPENONE FROM THE METHANOL ROOT EXTRACT OF *Cassia sieberiana* DC (Fabaceae)

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ABSTRACT

Cassia sieberiana DC (Fabaceae) is a small tree with wide spread in India and tropical Africa. The plant is used ethnomedicinally in African for the treatment of malaria, inflammations and other infectious diseases. Some of the pharmacological activities of the plant include antispasmodic, anti-inflammatory and antimicrobial activities.*β*-sitosterol and lupenone were isolated by a combination of column chromatography and gel filtration of ethylacetate fraction of the methanol root extract of the plant. The structures of the compounds were determined by analysis of their UV, IR, 1D and 2D NMR spectra as well as comparison with the literature data. This is the first report for the isolation of these compounds from the plant.

Keywords: *Cassia sieberiana*, β-sitosterol, lupenone, 1D and 2D NMR.

INTRODUCTION

Cassia sieberiana DC (Fabaceae)is a small tree with 15 to 20m tallness. It has short twisted bark with brownish grey colour and it has blackish stripes and young branches with densely leaves. It is a plant with wide spread in india and tropical Africa (Dalziel, 1956). It is one of those plants that possess medicinal properties which made it to be widely used in the treatment of various diseases and it is distributed all over Sudan Savanna, Senegal, Cameroon, Gambia, Congo, Uganda and Nigeria among others (Micheal 2014). Medicinal plants are known to be the rich source of raw materials used as traditional medicines in Africa and in other parts of the world. Several researches on medicinal plants preparations have led to the discovery of many potent drugs which are used today in modern clinical practices (Burkill, 2000).

The preliminary phytochemical analysis of the root extract of the plant revealed the presence of flavonoids, anthracenic derivatives and non-hydrolysable tannins. Previous studies on the ethanol root extract of *the plant have* shown anti-parasitic effect, myorelaxant and anti-spasmodic activity. (Fall *et al*., 2005).

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh roots of *Cassia sieberiana* were collected from Biye forest of Shika town, Giwa Local Government, Area-Kaduna State on April, 2018. The plant was

identifiedby MallamNamadiSunusi of the Herbarium Unit of Botany Department, Ahmadu Bello University, Zaria by comparing with the existing voucher specimen Number572.

Extraction and Preparation of the Plant Material

The roots were air dried and pounded into small size. The powdered root material (1.5 kg) was exhaustively extracted with 75% methanol using cold maceration method for 3 days which was filtered using whatch man No.1filter paper. A dark brown methanol root extract(12 g) was obtained after evaporation of the solvent. The extract was suspended on water to afford water soluble and insoluble portions. The water soluble portion was partitioned using n-hexane, chloroform, ethylacetate and n-butanol which affordedn-hexane, chloroform, ethylacetate and n-butanol fractions.

Isolation and Purification of pure compound

Based on the quantity and the resolution of the TLC profile of all fractions, ethlyacetate fraction (5g) was subjected to column chromatography over silica gel (mesh size 60-120) packed into 75 by 3.5cm column which was eluted using 100%n-hexane, followed by mixture of n-Hexane and ethylacetate (95:05, 90:10, 85:15, 80:20, 75:25, 70:30, 60:40, 50:50, 40:60 and 30:70)and it was finally washed with 100% ethylacetate, whch afforded eighty five fractions of 50mL each. These fractions were pooled together based on TLC profile similarity to give twenty five fractions coded A-Y and the column was finally washed with methanol to give the twenty sixth fraction coded (Z). Fraction G with two(2) distinct spots was subjected to repeated gel filtration which was eluted withmethanol over sephadex (LH-20). The process afforded 18 fractions of 2mL each. Fraction 13 gave a homogenous single spot on TLC plate and it is coded compound 1.Fraction 18 with one major spot and two minor spots was further purified by repeated gel filtration over these phadex L-H 20 which yielded single major spot on TLC plate in a range of 12-14 which was pooled and coded as compound 2. The two compounds were isolated from the same bulk fraction.

RESULTS AND DISCUSSION

Compound 1 (7.0 mg) was isolated as white crystalline: gave an R_f =0.48 using hexane and ethylacetate (8:2) as solvent system (Table 1). The^{1}H NMR spectrum of Compound 1 showed proton resonances between δ_H 0.5 and δ_H 2.5 representing overlapping methyl and methylene protons typical of stereoidal nucleous. (Sani *et al*.,2015). Signal atδ_H3.5 was due to methine proton at position 3,proton resonance at δ_H 5.8 was assigned to methine proton at position 6 while δc 11.8-56.7 represent a region of overlapping methyl, methylene and methine carbons; anoxymethine signal δC 71.8 of β -Sitosterol and unsaturated carbon resonance at δC 121.7 and δC 141.7 were assigned to a two carbon olefinic system. These NMR data of the compound was very similar to the reported data (Pateh *et al*., 2009; Hamada *et al*., 2012; Sani *et al*., 2015). Therefore, the structure of compound 1 was determined to be β- Sitosterol (Figure 1).

Table 1: Comparison of 1D spectral data for compound 1 with literature.

Position	$^{1}H^{1}H$ (compound 1)	$^{1}H^{1}H$ (Hamada *et al.*, 2012)	^{13}c (compound 1)	^{13}c (Hamada *et al.*, 2012)
1			37.5	37.4
2	1.56m	1.54m	40.0	40.0
3	3.50m	3.50m	72.1	72
4	2.32m	2.29m	42.6	42.5
5	5.33t	5.34t	141.7	140.9
6			121.7	121.9
7	2.04m	2.02m	32.2	32.1
8			31.9	31.9
9	1.55m	1.54m	50.4	50.3
10			36.8	36.7
11			21.3	21.3
12			40.0	40.0
13			42.6	42.5
14			57.0	57.0
15			26.4	26.3
16			28.5	28.4
17			56.3	56.2
18			36.4	36.3
19	0.19d	0.19d	19.3	19.2
20			34.2	34.1
21			24.5	24.5
22			46.1	46.0
C-23			23.3	23.2
C-24	0.82t	0.82t	20.0	20.0
C-25			29.4	29.3
C-26	0.81d	0.82d	20.0	20.0
C-27	0.79d	0.80d	19.6	19.6
C-28	0.66s	0.69s	19.0	19.0
C-29	0.99s	1.0s	12.1	12.0

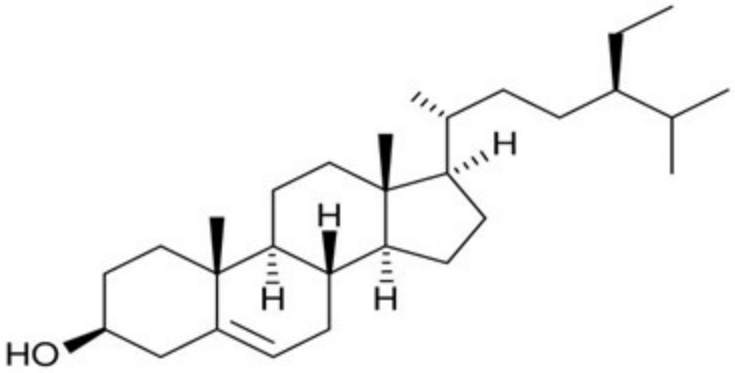

Figure 1: β- Sitosterol

Compound 2 was isolated as a white powder with the melting point within the range of 168-170°c. The ^{1}H NMR spectrum of compound 2 revealed seven methyl singlets atδ_H0.74, 0.78, 0.83, 0.91, 0.94, 1.06 and 1.72 while δ_H4.56 and 4.74 are proton signals representing the exocyclic double bond of the compound (Chaturvedula and prakash, 2012). The ^{13}C NMR spectrum of the compound showed a saturated carbonyl group at δ_C218.4 and the alkene carbons at δ c150.4 and δc108.8; suggesting the presence of a lupanetriterpene (Prakash *et al.,* 2014) (Table 2).The lupane skeleton for the compound and the absence of hydroxyl group suggested the presence of a keto functional groupat position C-3 identified by HMBC correlations,thus, the compound was found to belupenone which was further confirmed by comparison with the reported data (Chaturvedula and Prakash, 2012) (Figure 2).

Table 2: The comparison of 1D spectral data of compound 2 with literature

Position	**^{1}H(compound 2)**	**^{1}H-NMR (Chaturvedula e*t al.*, 2012)**	**^{13}C (Compound 2)**	**^{13}C (Chaturvedula e*t al.*, 2012)**
1			39.7	39.8
2			35.1	35.7
3			212.8	218.4
4			41.6	41.0
5			59.3	55.1
6			18.5	18.2
7			33.1	41.0
8			41.1	55.1
9			38.1	35.7
10				
11			22.5	21.7
12			29.4	30.0
13			35.6	35.7
14			41.4	41.0
15			30.2	30.0
16			36.0	35.7
17			42.2	43.1
18			53.1	
19	2.42-2.57m	2.42-2.52m	42.6	43.1
20			150.4	151.1
21			29.7	30.0
22			40.8	40.2
23	1.04(3H,s)	1.04(3H,s)	32.3	23.2
24	1.00(3H,s)	1.00(3H,s)	32.0	30.0
25	0.91(3H,s)	0.90(3H,s)	18.9	18.2
26	1.22(3H,s)	1.22(3H,s)	18.0	18.2
27	0.92(3H,s)	0.92(3H,s)	15.1	14.7
28	0.79(3H,s)	0.77(3H,s)	20.2	19.9
29	4.57(3H,s)	4.55(3H,s)	108.8	109.6
30	1.66(3H,s)	1.66(3H,s)	21.0	21.2

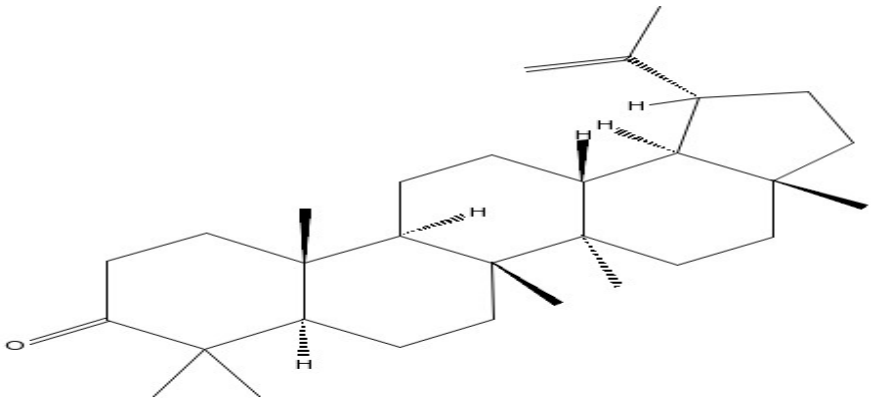

Figure 2: Lupenone

CONCLUSION

This study afforded β-sitosterol and lupenonefrom the root extract of *Cassia sieberiana* DC. To the best of this search, this is the first report for the isolation of the two compounds from the plant.

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