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ISOLATION OF SITOSTEROL AND STIGMASTEROL FROM THE ROOTS OF *CROTON PENDULIFLORUS*

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ABSTRACT

The primary aim of this study was to isolate Sitosterol from the root of Croton Penduliflorus. Croton Penduliflorus Hutch (Euphorbiaceae) commonly known as Turk's Cap though to originate from Malaysia, is a tropical evergreen plant widely distributed in the southern part of Nigeria. It is employed in folklore medicine both as drastic and fugitive, as well as a psychotropic medicinal plant. It is also credited with antimicrobial, antivenom, antiparalytical, rubefacient and anti-tumor potencies Extraction of the roots of Croton Penduliflorus with ethanol and partitioning of the extract with hexane, chloroform and ethyl acetate followed by column chromatography of the chloroform extract has led to the isolation of sitosterol, stigmasterol and a substituted benzene derivative. Their structures were established using 1D and 2D NMR, LCMS, and FTIR spectral analysis and a comparison of their spectral data with literature reports.

Keywords: *Croton Penduliflorus*, Stigmasterol, Sitosterol, Nuclear Magnetic Resonance

INTRODUCTION

In recent years, renewed interest has been shown in the use and efficacy of medicinal plants as a means of alleviating as well as treating specific disease conditions (Esther *et al.*, 2020). As a result of this, there has been increased awareness by the government scientific and medical communities of the importance of medicinal plants as a therapeutic and essential pivot in healthcare programmes especially in developing countries. Among the medicinal plant preparations in current and common use in Nigeria is the ground form of the seed of *Croton Penduliflorus* of the family of Euphorbiaceae. It is often used as a purging nut and possesses inflammatory, vesicant and contraceptive properties (Esther *et al.*, 2020). Natural products from medicinal plants, either as standardized extracts or as refined compounds provide limitless prospects for new drug leads due to their chemical diversity (John *et al.*, 2022) Drug discovery from medicinal plants based on ethnopharmacological practices or reported uses has gained prominence in recent times (John *et al.*, 2022, Ribeiro-Filho *et al.*, 2022, 2023). Other modern strategies involve the virtual or computer-aided screening of natural compounds in the form of libraries or isolated compounds for active moieties and to identify pharmacophores responsible for their activity (Najmi *et al.*, 2022). Natural product libraries or boxes containing a diversity of natural compounds are also being generated and compounds are being tested for activities based on the diverse chemical space that they inherently provide. Thus, the isolation of novel or known compounds to add to the compound libraries and introduce more chemical diversity is a new approach to drug discovery from natural products and testing for the toxicity of the natural compounds and their extracts. *Croton* species play relevant roles in the treatment of diseases by traditional healers and, as such, a comprehensive study of their chemical constituents is vital for validation of their traditional medicinal uses as well as exploring new potential sources of novel therapeutics. *Croton Penduliflorus* (Euphorbiaceae) has been found to indicate histopathological and gross changes in the duodenum, stomach, colon, and ileum (Asuzu *et al.*, 1988). It is also considered to possess anti-venom, antimicrobial, anti-paralytic, anti-tumor, and rubefacient activities (Tania *et al.*, 2020). The plant forms a significant component of abortifacients, herbal contraceptives, and anti-fibroid concoctions used in the local treatment of fibroids (Ojokuku *et al.*, 2010). Its wide use in traditional medicine has necessitated this study to isolate and characterize the chemical constituents.

MATERIALS AND METHODS

Plant Material

The roots of the plant were collected from a Savannah woodland, 11.0855°N, 7.7199°E, at Sakaru village, Zaria, Kaduna State. The plant was authenticated by Dr. U.S Gallah of the Department of Botany, Ahmadu Bello University, Zaria. A Voucher Specimen, ABU/H/13715 has been retained at the herbarium of the Department. The collected roots were cut into small pieces and then air-dried as described by Sylvester et al. (2022).

Extraction of Plant Material

The maceration method was adopted in which the air-dried, powdered root sample (325.0 g) was macerated thrice with ethanol (1.5 L) for three days at room temperature. The combined ethanol extract was evaporated under reduced pressure with a rotary evaporator that yielded 11.5 g of the solvent-free extract (Ojokuku *et al.*, 2010).

$$Yield = \frac{weight\ of\ dried\ oil}{weight\ of\ powdered\ sample} \times 100\%$$

Yield = 3.54%

Partitioning of the Extract

The extract was suspended in water (124 mL) and successively extracted with chloroform (3 x 124 mL), hexane (3 × 124 mL), and ethyl acetate (3 × 124 mL). The fractions were evaporated to dryness using a water bath and formed

1.10 g, 1.20 g and 1.20 g of the chloroform, hexane and ethyl acetate fractions respectively.

Qualitative Screening

The chloroform fraction was spotted on TLC plates and developed with chloroform: methanol in ratio 100:1, 80:1, 50:1. The developed plates were subsequently viewed under UV light (254 nm) and sprayed with a 10 % sulfuric acid in ethanol, followed by heating with hair-drier.

Isolation of Compounds

The extract was fractionated on a silica gel column (silica gel (60-200 mesh), 100 g) and eluted using chloroform and 80:1 $CHCl_3/CH_3OH$. A total of 5 eluates, each 30 ml, were collected for chloroform, and a total of 9 eluents, each 30 ml were eluted with 80:1 chloroform/methanol. The constituent profile of each eluate was monitored by TLC (100% $CHCl_3$ and 80:1 $CHCl_3/CH_3OH$) and visualized using a UV-lamp at 254 nm and using spray reagent (10 % sulfuric acid in ethanol and heating). Based on their TLC profile, the eluates were combined and labeled CPC-3A, CPC-3B, and CPC-5 (Ijeoma *et al.,* 2017).

Characterization

Structures were established using spectroscopic methods. Isolated compounds were sent to the Royal Botanic Garden, Kew for 1D and 2D NMR, LCMS, and FTIR analysis. The NMR experiments were conducted on a 500 MHz Bruker AVANCE III spectrophotometer. Spectra were recorded in $CDCl_3$ and referenced to the solvent residual signal at δ_H 7.26 in the ^{1}H-NMR and at δ_C 77.23 in the ^{13}C-NMR. The spectra were processed using Bruker NMR academic Topspin software. LC-MS analysis was carried out using an Agilent Technologies 6890 N and GC system coupled to an Agilent Technologies HP5973 mass electron detector with samples dissolved in CH_2Cl_2. Infra-red (IR) spectra were recorded in the range 600-400 cm^{-1} using a Perkin Elmer Spectrum Two FT-IR Spectrometer.

RESULTS AND DISCUSSION

Identification of CPC–3A as sitosterol (1)

Compound CPC–3A was isolated as a white crystalline solid from the ethanol extract of the roots of *Croton penduliflorus*. It was identified to be the known phytosterol, sitosterol which has been previously isolated from several African *Croton* plants, including *C. mubango*, *C. sylvaticus*, and *C. haumanianus*. (Isyaka *et al*., 2020).

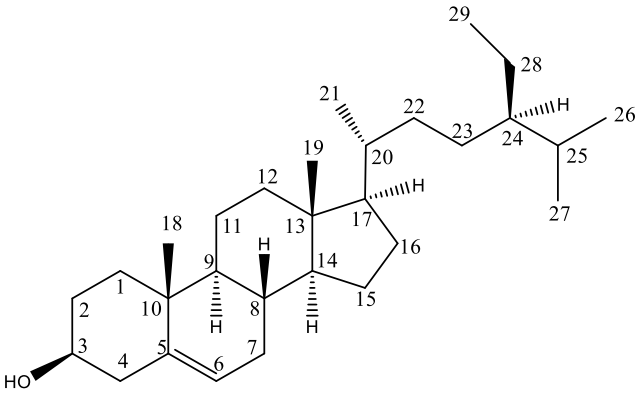

Figure 4: Sitosterol

The LCMS spectrum for compound **1** gave a molecular ion peak at m/z 414, indicating $C_{29}H_{50}O$ molecular formula, corresponding to five degrees of unsaturation. The FTIR spectrum showed absorption bands at 3414 cm^{-1} for the hydroxyl group stretching (Silverstein *et al.,* 1997). The ^{1}H-NMR spectrum displayed proton resonances at δ_H 5.35 (m), assigned to H-6. The oxymethine proton resonance at 3.52 (m) was assigned to H-3. The ^{13}C-NMR data of Compound **1** were compared to those reported by Kojima et al., (1990) for sitosterol and were found to agree (Table 1).

Table 1: NMR data of compound CPC-3A: Sitosterol compared against literature reference values.

No.	^{13}C-NMR (125 MHz) in $CDCl_3$	^{13}C-NMR (67.9 MHz) in $CDCl_3$ (Kojima *et al,* 1990)	^{1}H-NMR (500 MHz) $CDCl_3$ (*J* in Hz)
1α	37.5 CH_2	37.2	1.84 m
1β			1.06 m
2α	31.9 CH_2	31.6	1.84 m
2β			1.50 m
3	72.1 CH	71.8	3.52 m
4α	42.5 CH_2	42.3	2.25 m
4β			2.25 m
5	141.0 C	140.7	-
6	122.0 CH	121.7	5.35 m
7α	32.1 CH_2	31.9	1.96 m
7β			1.96 m
8	32.1 CH	31.9	1.49 m
9	50.3 CH	50.1	0.91 m
10	36.7 C	36.5	-
11α	21.3 CH_2	21.1	1.47 m

11β			1.47 m
12α	40.0 CH_2	39.8	2.00 m
12β			1.15 m
13	42.5 C	42.3	-
14	57.0 CH	56.8	0.98m
15α	24.5 CH_2	24.5	1.58 m
15β			1.08 m
16α	28.5 CH_2	28.2	1.83 m
16β			1.83 m
17	56.3 CH	56.0	1.09 m
18	12.1 CH_3	11.9	0.84 s
19	19.6 CH_3	19.4	1.00 s
20	36.4 CH	36.1	1.35 m
21	19.0 CH_3	18.8	0.82 s
22α	34.2 CH_2	33.9	1.32 m
22β			1.01m
23α	26.3 CH_2	26.0	1.15 m
23β			1.15 m
24	46.0 CH	45.8	0.92 d *J* = 6.5
25	29.4 CH	29.1	1.65 m
26	20.0 CH_3	19.8	0.84 s
27	19.3 CH_3	19.0	0.80 s
28α	23.3 CH_2	23.0	1.25 m
28β			1.25 m
29	12.2 CH_3	12.0	0.66 dd *J* = 1.3, 8.0

Identification of compound 2 as stigmasterol

Compound 2 was isolated as a white crystalline compound from the ethanol extract of the roots of *Croton penduliflorus* and was identified to be stigmasterol, previously isolated from *Croton pseudopulchellus*.

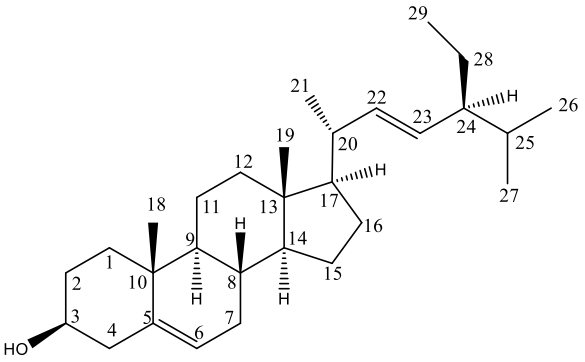

Figure 5: Stigmasterol

The LCMS spectrum for compound **2** gave a molecular ion peak at *m/z* 412, which indicated a molecular formula of $C_{29}H_{48}O$, corresponding to six degrees of unsaturation. The FTIR spectrum for compound **2** displayed an absorption band at 3401 cm^{-1} that was ascribed to a hydroxyl stretch and absorption bands at 2932 cm^{-1} and 2867 cm^{-1} that were attributed to sp^3 C-H stretches (Silverstein *et al*., 1997). The ^{1}H-NMR spectrum displayed proton resonances at δ_H 5.13 (dd, *J* = 8.5, 15.2 Hz), 4.99 (dd, *J* = 8.5, 15.2 Hz), 5.32 (d, *J* = 5.0 Hz) for double bond proton resonances. An oxymethine proton resonance at δ 3.49 (m) was also observed for Compound CPC – 3B. The ^{13}C-NMR spectrum for compound **2** showed 29 carbon resonances which were comparable to those reported by **(**Anderson *et al.,* 1975) for stigmasterol as presented in Table 2. Therefore, it was identified as stigmasterol.

Table 2: NMR data of compound CPC-3B: Stigmasterol compared against literature reference values.

No.	**^{13}C-NMR (125 MHz) in $CDCl_3$**	**^{13}C-NMR (125 MHz) in $CDCl_3$** (Anderson *et al.,* 1975)	**^{1}H-NMR (500 MHz) $CDCl_3$ (*J* in Hz)**
1α	37.5 CH_2	37.2	1.81 m
1β			1.34 m
2α	31.9 CH_2	31.6	1.95 m
2β			1.43 m
3	72.0 CH	71.8	3.49 m
4α	42.5 CH_2	42.3	2.24 m
4β			2.24 m

5	141.0 C	140.7	-
6	121.9 CH	121.7	5.32 d *J* = 5.0
7α	32.1 CH_2	31.9	1.81 m
7β			1.48 m
8	32.1 CH	31.9	1.41 m
9	50.3 CH	50.1	0.90 m
10	36.7 C	36.5	-
11α	21.3 CH_2	21.1	1.45 m
11β			0.78 m
12α	40.7 CH_2	39.7	1.97 m
12β			1.14 m
13	42.5 C	42.3	-
14	57.1 CH	56.9	0.97 m
15α	24.5 CH_2	24.3	1.56 m
15β			1.05 m
16α	29.1 CH_2	29.0	1.23 m
16β			1.13 m
17	56.3 CH	56.0	1.08 m
18	12.2 CH_3	12.0	0.65 s
19	19.6 CH_3	19.4	0.81 s
20	40.7 CH	40.5	1.14 m
21	21.3 CH_3	21.1	0.99 s
22	138.6 CH	138.3	5.13 dd *J* = 8.5, 15.2 Hz
23	129.5 CH	129.25	4.99 dd *J* = 8.5, 15.2 Hz
24	51.5 CH	51.2	1.50 m
25	32.1 CH	31.9	1.48 m
26	21.4 CH_3	21.2	0.79 s
27	19.2 CH_3	19.0	0.79 s
28α	25.6 CH_2	25.4	1.21 m
28β			1.06 m
29	12.6 CH_3	12.2	0.65 dd *J* = 1.3, 8.0

CONCLUSION

Isolation of active compounds from the Chloroform extract fraction of roots of *Croton penduliforus* gives CPC- 3A, CPC-3B and CPC- 5. Structural elucidation based on spectral data suggested that the CPC- 3A, CPC- 3B and CPC- 5 are Sitosterol, Stigmasterol and fatty acid respectively. Furthermore, it is essential to fully characterize CPC-5.

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