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Phytochemical and Structure Elucidation of Stigmasterol from the Stem Bark of *Pseudocedrela kotschyi* (Harms) (Meliaceae)

V. Atinga[1*], A. Ahmed[2], H. Nuhu[2], E. A. Ayeni[2] and G. Dauda[3]

[1]Department of Pharmacognosy and Drug Development, Gombe State University, Tudun Wada, Gombe, Nigeria.
[2]Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria.
[3]Department of Pharmaceutical Chemistry, Ahmadu Bello University, Zaria, Nigeria.

This work was carried out in collaboration between all authors. Authors AA and HN designed the study. Author VA sourced the plant material used and Authors VA and EAA performed all experiments. Author GD carried out interpretation of spectra. Authors VA and GD managed the literature searches. Author VA wrote the first draft of the manuscript. All authors read, corrected and approved the final manuscript.

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ABSTRACT

Aim: To perform phytochemical screening, isolate and characterize components from the n-hexane extract of *Pseudocedrela kotschyi* stem bark.
Methodology: The stem bark of *P. kotschyi* was collected, prepared and exhaustively extracted using analytical graded n-hexane and phytochemical constituents were determined according to the standard method. The Column chromatography technique was used to isolate and purify compound from n-hexane extract using gradient elution techniques. The isolated compound (PKV) obtained was subjected to physical, chemical and spectral analysis by UV, IR, 1D and 2D NMR.
Results: The Phytochemical screening revealed the presence of steroid/triterpenes and cardiac glycosides. The phytochemical investigation of the hexane extract of *Pseudocedrela kotschyi* led to

**Corresponding author: E-mail: pharmvalat484@gmail.com;*

the isolation of stigmasterol from the stem bark. The structure was ascertained by extensive comparison of the 1D & 2D NMR data and with their physical appearance with that reported in literature earlier.
Conclusion: To the best of our knowledge, this is the first report on the isolation of this compound from this plant.

Keywords: Pseudocedrela kotschyi; stem bark; phytochemical screening; NMR; hexane.

1. INTRODUCTION

In recent time, there has been a growing interest in natural product research due to the failure of alternative drug discovery methods to deliver many lead compounds in critical therapeutic areas such as immunosuppression, anti-infectives, and metabolic diseases [1]. It has been estimated that about 40% of all medicines are either natural products or semi-synthetic derivatives [2]. The plant *Pseudocedrela kotschyi* (Harms) is a small deciduous tree that belongs to the Meliaceae family. It is widely spread in the savanna zone from east Senegal to western Ethiopia, Uganda and Nigeria. It is commonly called dry-zone cedar and hard cedar-mahogany, and locally is known as *Emi gbegi* among Yoruba's and *Tuna* among Hausa's. The decoction of the leaf is used traditionally in the folk medicine in Nigeria for the treatment of many diseases and health conditions, including, fever, pains, diabetes and convulsion [3-6]. The roots and leaves are used to treat rheumatism and dysentery [7]. The plant is used traditionally in Ghana to treat leprosy and epilepsy [8], malaria and stomach aches [9-11]. The preliminary phytochemical screening of the methanol extract revealed the presence of saponins, flavonoids, tannins, glycosides, anthraquinones, steroids/terpenes and alkaloids. Some of the chemical constituents of *P. kotschyi* isolated include limonoids, 7-desacetoxy-7- oxogedunin and pseudrelones A, B and C which displayed good antiprotozoal activity [12]. The aqueous stem bark extract was investigated to have antiulcer activity [13]. The n-butanol soluble portion of the ethanolic extract of the leaves of *P. kotschyi* has been shown to possess anti-nociceptive and anti-inflammatory activities in mice and rats respectively [14]. *Pseudocedrela kotschyi* root extracts have been shown to inhibit the *in vitro* growth and development of the schizont stage of *Plasmodium falciparum* [15]. Only a few phytochemicals responsible for the activities of this plant have been isolated and characterized. This study aims to carry out phytochemical analysis and to isolate and elucidate compounds from *Pseudocedrela kotschyi* stem bark.

2. MATERIALS AND METHODS

2.1 Plant Material and Collection

The stem bark of *Pseudocedrela kotschyi* was collected around March 2017 in Dajin Kudingi area, Samaru district of Sabon Gari Local Government, Kaduna State. The plant was identified by Malam Namadi Sanusi at the Department of Botany, Ahmadu Bello University, Zaria-Nigeria with a voucher number 900243. A voucher specimen was deposited for future reference.

2.2 Preparation of Plant Sample

The Stem bark of the plant was cleaned, air dried and ground to coarse powder using grinding machine. The powder was stored in airtight containers for further use.

2.3 Extraction of Plant Materials

Stem bark powder of *Pseudocedrela kotschyi* (1.2 kg) was exhaustively extracted in 2.5 litres analytical graded n-hexane according to the standard method for 72 hours using cold maceration and the concentrate was evaporated to dryness on a water bath and stored in the desiccator for further use.

2.4 Phytochemical Screening

The n-hexane extract was subjected to phytochemical screening using standard method as described by Evans, 2008 and Sofowora, 1993 [16, 17] to detect the presence or absence of chemical constituents such as carbohydrate, cardiac glycosides, saponins, alkaloids, triterpenes/steroids, tannins, anthraquinones and flavonoids.

2.5 Isolation of Compound from Hexane Extract

Column chromatography of *n*-hexane extract (3.0 g) was conducted using silica gel (Mesh 60-120 mm) that was packed using wet packing method in *n*-hexane. The column was eluted using solvent systems with increasing polarity starting with *n*-hexane (100%) then *n*-hexane: ethyl acetate in varying ratios by using gradient elution technique and a total of 135 eluates were collected and concentrated. TLC pre-coated silica gel plates sprayed with *p*-Anisaldehyde sulphuric acid reagent and heated at 110^0 C were used to monitor the homogeneity of the eluates. Similar fractions were pooled together. Pooled fractions 75-80 were fractioned in a mini column filled with silica gel 86 (60- 120 mm) to obtain a pure compound.

2.6 Physical and Chemical Analysis

Physical study was conducted on the isolated compound to determine its melting point (Chemiline-715 melting point apparatus), color and solubility in different solvents (hexane, chloroform, Ethylacetate and methanol). Chemical test was also conducted to ascertain the purity and nature of compound by using the following reagents; Libermann- Burchard, $FeCl_3$ and Dragendorf's, Salkwoski.

2.7 Spectroscopic Analysis

IR measurements were obtained on Perkin-Elmer (FT-IR) infrared spectrophotometer and UV-visible absorption was obtained using absorption spectrometer at Multiuser Research Laboratory, Department of Chemistry Ahmadu Bello University Zaria. 1H-NMR and 13C-NMR(1D and 2D) spectra were done in CDCl3 with NMR spectrometer and were recorded on Bruker DRX 300, 400 MHz ((Bruker Bio Spin, Rheinstetten, Germany) at Natural Product Laboratories, Strathclyde Institute of Pharmacy and Biomedical Sciences (SIPBS), University of Strathcyde Glasgow, United Kingdom. The chemical shifts (δ) were reported in parts per million (ppm) and the coupling constants (J values) were reported in hertz.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical screening of the hexane extract revealed the presence of steroids, triterpenes and cardiac glycosides (Table 1).

3.2 Preliminary Studies on the Pure Compound Isolated

The preliminary studies conducted on the compound PKV include colour appearance, solubility test, melting point determination and chemical test. The outcome obtained for each analysis done is shown in Table 2.

Table 1. Phytochemical constituents present in the hexane extract of *P. kotschyi*

S/no.	Phyto constituents	Status
1	Saponins	-ve
2	Flavonoids	-ve
3	Triterpenes	+ve
4	Steroids	+ve
5	Tannins	-ve
6	Alkaloids	-ve
7	Cardiac Glycosides	+ve

Key: +ve means present and -ve means absent

Table 2. Physical and chemical studies parameters of the pure compound (PKV) isolated

Preliminary study	Parameter
Colour	White amorphous powder
Solubility	Partially soluble in Hexane Completely soluble in Chloroform
Melting point	104-106^0 c
Chemical test	Positive to Libermann Buchard reagent with green colour
R_f values	0.62 = Hexane: ethyl acetate (8:2) 0.77 = Hexane: ethyl acetate (7:3) 0.86 = Hexane: ethyl acetate (6:4)

The phytochemical screening of the hexane extract revealed the presence of steroids, triterpenes and cardiac glycoside. Other phytochemicals like alkaloid, flavonoid, anthaquinone and phenols were absent because hexane is a highly non-polar solvent and has more affinity for non-polar phytochemicals thereby extracting them as seen in Table 1.

The column chromatography of the hexane extract led to the isolation of a white amorphous powder compound coded PKV. The isolated compound PKV gave a single homogenous spot with three different TLC solvent systems indicating the purity of the compound with Rf of 0.62, 0.77 and 0.86. The melting point of 104-106° c further suggested that the compound was a steroid [18]. It was completely soluble in chloroform but partially soluble in hexane. The UV absorption maxima at 257nm indicate

the presence of chromophore in the compound PKV.

The IR spectrum showed absorption bands at 3429.2 cm-1 is due to free OH, 2933.4cm-1 is due to C-H stretch of CH_2 (asymmetrical) of an alkane, 2866.3 cm-1 is due to C-H stretch of a CH_3 (symmetrical bending). Absorption bands at 2070.1 cm-1 and 1915.9 cm-1 are due to overtone bands, 1461.1 cm-1 is due to sp bending and 1379.1 cm-1 is due to sp3 bending. Absorption bands at 1051.1 cm^{-1} are due to c-c-o stretch of a cyclic compound and 738.0 cm-1 is due to a tri-substituted carbon. The absorption frequencies resemble those of stigmasterol [19].

The 1H NMR spectrum showed a doublet of doublet at δH 3.52 (1H, d, H3) and also revealed the presence of three (3) olefinic protons at δH 5.36 (1H, d), δH 5.16 (1H, dd, H20) and δH 5.0 (1H, dd, H21) [20] and [21]. Protons signal at δH 0.72 (s, 3H, H18) and δH 1.01 (s, 3H, H19) are due to angular methyl proton [20] and [21].

The 13C NMR spectral analysis revealed the presence of 29 carbons signal due to triterpenoid nucleus [20] and [21]. The chemical shift at δC3 71.79 is due to oxymethine carbon, signals at δC 140.86 and δC 121.87 are due to double bond between C5 and C6 respectively [20] and [21]. Carbon signals at δC 138.23 and δC 129.16-129.60 indicates carbon in a trans conformation of a triterpenoid nucleus. Carbon signal at δC 43.16 is due to the influence of germinal dimethyl carbons.

Table 3. Summary of 1D and 2D spectral data for compound PKV in $CDCL_3$ (400MHz)

Position	δ H	δ ^{13}C	APT	COSY	HSQC	HMBC
1	1.08 (m, 1H)	36.67	CH_2			C-5, 9, 10
2	1.45 (m, 1H)	31.41	CH_2	H-1a	1.83#31.41	C-3, 5
	1.83 (m, 1H)					
3	3.52 (dd, 1H)	71.97	CH	H-4b, H-2b	3.52#71.97	
4	2.26(m, 1H)	42.47	CH_2		2.26#42.27	C-3, 5, 6
	2.31 (m, 1H)					
5	-	140.86	C			
6	5.36(d, 1H)	121.87	CH	H-4, H-7		
7	1.45 (m, 1H)	31.41	CH_2	H-15b		
	1.99 (m, 1H)					
8	1.38 (m, 1H)	34.16	CH			
9	0.93 (m, 1H)	50.30	CH			
10	-	36.30	C			
11	1.45(m, 1H)	21.24	CH_2		1.45#21.24	
12	1.19 (m, 1H)	37.33	CH_2	H-14, H-15b	1.19#37.33	
	2.03 (m, 1H)					
13	-	46.00	C			
14	1.03 (m, 1H)	56.93				
15	1.03 (m, 1H)	23.84	CH_2			
	1.56 (m, 1H)					
16	1.27 (m, 1H)	29.19	CH_2		1.27#29.9	
	1.79 (m, 1H)					
17	1.16 (m, 1H)	56.22	CH		1.16#56.22	C-18
18	0.72 (s, 3H)	12.92	CH			
19	1.01 (s, 3H)	19.55	CH_3			
20	2.09 (m, 1H)	39.94		H-16, H-17	2.09#39.94	
21	1.03 (s, 3H)	21.24	CH_3			
22	5.16 (dd, 1H)	138.46	CH	H-20		
23	5.02 (dd, 1H)	129.43	CH	H-25, H-20		
24	1.50 (m, 1H)	52.31	CH	H-29		
25	1.52 (m, 1H)	43.16	CH			
26	0.91 (m, 3H)	21.24	CH_3			
27	0.82 (trs, 3H)	19.20	CH_3			C-20, 26, 29
28	1.25 (m, 1H)	26.24	CH_2	H-29		C-29
	1.56 (m, 1H)					
29	0.83 (trs, 3H)	12.12	CH_3			C-27

Table 4. 1H and 13C NMR Chemical Shift Data of Compound PKV and Literature in CDCl3 (400MHz)

Carbon atom	δ ^{13}C NMR PKV	δ ^{13}C NMR Luhata et al, 2015	δ H NMR	δ H NMR Luhata et al, 2015
1	36.67	37.15	1.08 (m, 1H)	
2	31.41	31.56	1.83 (m, 1H)	
3	71.97	71.71	3.52 (dd, 1H)	3.51 (tdd, 1H)
4	42.47	42.19	2.26 (m, 1H)	
			2.31 (m, 1H)	
5	140.86	140.81	-	
6	121.87	121.62	5.36 (d, 1H)	5.31 (t, 1H)
7	31.41	31.56	1.45 (m, 1H)	
			1.99 (m, 1H)	
8	34.16	31.79	1.38 (m, 1H)	
9	50.30	50.02	0.93 (m, 1H)	
10	36.30	36.16	-	
11	21.24	21.12	1.45(m, 1H)	
12	37.33	39.57	1.19 (m, 1H)	
			2.03 (m, 1H)	
13	46.00	42.10	-	
14	56.93	56.76	1.03 (m, 1H)	
15	23.84	24.27	1.03 (m, 1H)	
			1.56 (m, 1H)	
16	29.19	28.83	1.27 (m, 1H)	
			1.79 (m, 1H)	
17	56.22	55.84	1.16 (m, 1H)	
18	12.92	12.15	0.72 (s, 3H)	1.03 (s, 3H)
19	19.55	19.88	1.01 (s, 3H)	0.71 (s, 3H)
20	39.94	40.40-40.51	2.09 (m, 1H)	
21	21.24	20.99	1.03 (s, 3H)	0.91 (d, 3H)
22	138.46	138.23	5.16 (dd, 1H)	4.98 (m, 1H)
23	129.43	129.16-129.60	5.02 (dd, 1H)	5.14 (m, 1H)
24	52.31	51.13-51.30	1.50 (m, 1H)	
25	43.16	31.94	1.52 (m, 1H)	
26	21.24	21.23	0.91 (m, 3H)	0.80 (d,3H)
27	19.20	19.01	0.82 (trs, 3H)	0.82 (d, 3H)
28	26.24	25.40-25.50	1.25 (m, 1H)	
			1.56 (m, 1H)	
29	12.12	12.25-12.30	0.83 (trs, 3H)	0.83 (t, 3H)

The utilization of the 1D and 2D NMR spectra and comparison with available literature data allowed us to propose the structure of PKV as stigmasterol

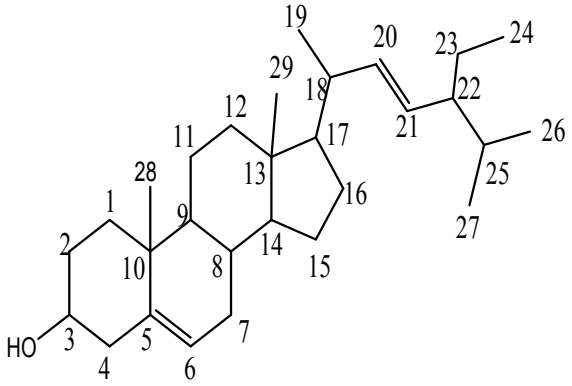

Fig. 1. Proposed structure of compound PKV

The APT experiment further confirmed the multiplicities of carbons which revealed 3 quaternaries (C), 11 methine (CH), 9 methylene (CH_2) and 6 methyl (CH_3) carbon. With the APT, it further supported the total number of carbons in the 13C NMR of the isolated compound (Table 3).

The COSY correlation spectra of compound PKV established the correlations between the protons such as 3.52 (H3) and 2.31 (H4b), 3.52 (H3) and 1.83 (H2b), 5.36 (H5) and 1.45 (H7), 1.25 (H28) and 0.83 (H29) among others (Table 3).

Protons were assigned to their respective carbons using HSQC. Among these attachments are δH 1.83 # δC 31.41 (H2# C2); δH 3.52 # δC 71.97 (H3 # C3); δH 2.26 # δC 42.27 (H4 # C4); δH 1.45 # δC 21.24 among others (Table 3).

The Heteronuclear Multiple Bond Correlation (HMBC) Spectroscopy was used to establish various linkages between the fragments. The HMBC spectra showed a long range correlation between δH 1.08 (H1) and δC 5,9,10 (140.56, 50.30, 36.3), 1.83 (H2) and δC3,5 (71.97, 140.56) and 2.26 (H4) and δC3,5,6 (71.97, 140.56, 121.87), 1.16 (H17) and δC18 (12.92) among others (Table 3).

4. CONCLUSION

The white amorphous powder that was successfully isolated from the hexane extract of the stem bark of *Pseudocedrela kotschyi* was found to be stigmasterol. To the best of our knowledge, this is the first report of the isolation of stigmasterol from the stem bark of *Pseudocedrela kotschyi.*

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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