Majalah Obat Tradisional (Trad. Med. J.), January-April 2024
Vol. 29(1), p 46-53
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 04-09-2023
Revised : 25-11-2023
Accepted : 13-03-2024

Characterization and Identification of Novel Steroids from *Nauclea pobeguinii* Leaves

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ABSTRACT

Nauclea pobeguinii is a plant in the *Rubiaceae* family endemic to the swamp forest region of the world. Its extract is widely used in traditional medicine for the treatment of a wide variety of ailments such as malarial, Jaundice, gonorrhea, fever, and stomach discomfort. While other parts of the plant have been examined for the bioactive principles responsible for the medicinal properties, limited information is available in the literature as regards the leaves, hence this study. *N. pobeguinii* leaves were collected, air-dried, and pulverized. The pulverized sample was extracted with solvents (*n*-hexane, ethyl acetate, and ethanol) of varying polarity to obtain the crude extracts. Repeated column and thin layer chromatographic separation of the crude extracts afforded three compounds, which were characterized by their IR, ^{1}H, ^{13}C-NMR, and 2D-NMR spectral data. A comparison of the data with literature confirmed the compounds to be 2-hydroxylstigmastane acetate (1), Ergosta-5,6-epoxy-22-en-3-yl-acetate (2), and β-sitosterol (3). Compounds 1 and 2 are novel to *N. pobeguinii*.

Keywords: 2-hydroxylstigmastane acetate; *Nauclea pobeguinii;* purification; *Rubiaceae*

INTRODUCTION

The use of medicinal plants to combat diseases and ailments is regaining momentum, especially in developing and underdeveloped countries of the world. This is due to their perceived safety, affordability, and easy access when compared to conventional drugs (Islam & Lucky, 2019). According to the World Health Organization (WHO), 80% of the world's population uses herbs to treat a variety of diseases as well as other healthcare requirements (Ribeiro et al., 2017; Samoisy & Mahomoodally, 2015).

The genus *Nauclea* and other species of the family *Rubiaceae* is the largest family of woody plants with over 13,000 species, and they belong to the *Cinchonoideae* sub-family. They are commonly found in tropical regions of the world, including Africa and Asia (Haudecoeu et al., 2018). *Nauclea pobeguinii (Hua ex Pobég) Merr* is a deciduous shrub native to swamp forests around the world. It is also known locally as *Opepe ira* in Western Nigeria. The plant extract is used in the treatment of Jaundice, gonorrhea, fever, stomach discomfort, and epilepsy, among others (Ambe et al., 2015; Njoya et al., 2017; Mac Donald and Olorunfemi, 2000; Mesia et al., 2005). The water decoction of the stem bark has also been reported in antihelminthic treatment (Bett, 2002; Luzakibanza, 2012; Mesia et al., 2005). Preparations from the stem bark of *Nauclea pobeguinii* also had analgesic, anti-inflammatory, and anti-arthritic (monoarthritis) properties (Mbiantcha et al., 2018).

Extracts of this plant are very rich in secondary metabolites, such as tannins, flavonoids, steroids, saponins, alkaloids, and terpenoids (Haudecoeu et al., 2018; Adepoju et al., 2020). Previously isolated compounds from *Nauclea pobeguinii* include strictosidine, naucleidinal, naucleofficine, kelampayoside, magniflorine, Augustineone (Xu et al., 2012), β-sitosterol, 7-hydroxystigmast-22-en-3.6-dione, and 3β, 24(S)-dihydroxycholesta-5, 25-dien-7-(Adepoju et al., 2022). In continuation of our search for new bioactives from this plant, we hereby report two novel steroids and another known one in this study.

MATERIALS AND METHODS

Collection and preparation of plant material

The leaves of *Naucleapobeguinii* were collected from the Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State, South West, Nigeria. It was identified and authenticated with the herbarium number FHI 108529. The plant sample was air-dried, after which it was milled into powder with the aid of an electrical grinder and finally stored in a moisture-free en m vironment.

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DOI: 10.22146/mot.88673 | *Majalah Obat Tradisional, 29(1), 2024*

Extraction of Phytoconstituents

Extraction was performed by continuously soaking the dried ground leaves (1333.5 g) with a range of solvents: *n*-hexane (5.5 L), ethyl acetate (5.0 L), and ethanol (4.5 L). Sequential extraction was adopted by using *n*-hexane first to remove non-polar organic compounds, waxes, and fats. The extract was filtered out after one week, and the residual plant sample was extracted with ethyl acetate and ethanol, respectively, to remove more polar compounds. Each extract obtained was concentrated in vacuousing a rotary evaporator. After the solvent had been removed, gumming solids were obtained. The weight of each extract was recorded for the crude extract of hexane, ethyl acetate, and ethanol (Das et al., 2010; Hesham et al., 2016).

Isolation of Phytoconstituents from *the crude extracts*

Ten grams (10 g) of ethanol leaves crude extract (ETL) obtained from the leaves of *N. pobegunii* was chromatographed in a silica gel column and eluted with a mobile phase of different polarities and strength; n-hexane 100 %, Hex/$CHCl_3$ 9:1, Hex/$CHCl_3$ 8:2, Hex/$CHCl_3$ 7:3, Hex/$CHCl_3$ 6:4, Hex/$CHCl_3$ 5:5, Hex/$CHCl_3$ 4:6, Hex/$CHCl_3$ 3:7, Hex/$CHCl_3$ 2:8, Hex/$CHCl_3$ 1:9, $CHCl_3$ 100 %, $CHCl_3$/MeOH 95:5, $CHCl_3$/MeOH 9:1, $CHCl_3$/MeOH 8:2, $CHCl_3$/MeOH 7:3, $CHCl_3$/MeOH 6:4, $CHCl_3$/MeOH 5:5, $CHCl_3$/MeOH 4:6, $CHCl_3$/MeOH 3:7, $CHCl_3$/MeOH 2:8, $CHCl_3$/MeOH 1:9 and MeOH 100 %. Equal volume of eluates (100 mL) was collected for each fraction, concentrated in vacuo, and analyzed for purity using analytical TLC to give a total of 97 fractions. Fractions with similar compositions were combined. Fractions 28-32 gave a single spot on the TLC plate with solvent system $CHCl_3$ 100%when viewed under the UV lamp at 254 nm.The fractions were combined and coded as $ETL1_{28\text{-}32}$.It formed white powder on evaporation, and the isolated compound was labeled as compound 1and the spectroscopic data was recorded. The data suggested compound 1 to be 2-hydroxylstigmastane acetate.

Fraction $ETL2_{33\text{-}46}$ of the above fractionation of the crude ethanol extract of leaves of *N. pobeguinii* were combined and chromatographed using hexane and chloroform (Hex/$CHCl_3$ 1:9, $CHCl_3$ 100 %) and assigned as $ETL_{33\text{-}46}$. Ten fractions of 30 ml each were afforded, and fractions 4-6 (100 % chloroform) were combined because of a similar TLC profile and coded as $ETL2_{2\text{-}4}$. Fraction $ETL2_{2\text{-}4}$ was further purified using a smallercolumn (25 cm x 2.1mm). This fractionation also afforded 14 fractions of 30 ml each with mobile phase (Hex/$CHCl_3$ 1:9, $CHCl_3$ 100 %). Fraction 3 gave two spots on the TLC plate, which were very close and coded as $ETL2_3$. Further purification of $ETL2_3$ with a smaller silica gel column (25 cm x 2.1mm) was done by eluting with mobile phase Hex/$CHCl_3$ 1:9, $CHCl_3$ 100 % and coded as ETL_3. Fourteen fractions were afforded. Fraction 9 gave a single spot on the TLC plate when viewed under UV light (254 nm). The isolated compound was obtained as a white powder assigned $ETL2_9$ and labeled as compound 2. The spectroscopic data was recorded. The spectroscopic data suggested compound 2 to be Ergost-5,6-epoxy-22-en-3-yl-acetate.

Fractions $ETL3_{62\text{-}66}$ of the above fractionation from ethanol crude extract of leaves of *N. pobegunii* was further chromatographed with a smaller silica gel column (25 cm x 2.1mm) and eluted with a mobile phase of increasing polarity and strength represented as follows;

Hex/$CHCl_3$ 8:2, Hex/$CHCl_3$ 7:3, Hex/$CHCl_3$ 6:4, Hex/$CHCl_3$ 5:5, Hex/$CHCl_3$ 4:6, Hex/$CHCl_3$ 3:7, Hex/$CHCl_3$ 2:8, Hex/$CHCl_3$ 1:9, $CHCl_3$ 100 %. This gave 20 fractions of 30 ml each, and fraction number 6 formed white powder, which gave a dark blue spot on the TLC plate with solvent system Hex/$CHCl_3$ 3:7 when viewed at 254 nm. The isolated compound was assigned $ETL3_6$ and labeled as compound **3**. The spectroscopic data suggested compound 3 to be β-sitosterol.

RESULTS

Three compounds were successfully isolated from the ethanol stem bark extracts of *N. pobeguinii*. Structural elucidation of the compounds was achieved through the spectroscopic data, i.e., FTIR, ^{1}H-NMR, ^{13}C-NMR, DEPT-135, COSY, HSQC, and a comparison of the spectral data with those reported in the literature. The spectral are presented as supplementary data. The nmr data are also summarized in Tables I & II.

Compound 1 was obtained as white powder with Rf value of 0.4 ($CHCl_3$ 100%). IR U (cm^{-1}, ATR) : 3415, 2922, 2848, 1729, 1460, 1386, 1251, 1073, 789. ^{1}H NMR δ (500MHz, $CDCl_3$): 3.99 (d), 3.75, 0.6-2.20 (s). ^{13}C NMR δ (500MHz, $CDCl_3$) 39.73 , 64.14, 70.77, 27.92, 42.73, 28.20, 32.74, 35.54, 45.84, 30.17, 21.08, 39.10, 45.34, 56.13, 24.13, 29.70, 55.34, 12.14, 19.82, 38.07, 22.67, 34.75, 26.58, 36.81, 31.93, 18.60, 18.24, 25.04, 11.96, 173.78, 21.09.

Compound 2 was obtained as white powder, (R_f value of 0.6 ($CHCl_3$ 100%) IR (cm^{-1}, KBr): 3415, 2922, 2848, 1729, 1565, 1460, 1162, 1073, 789. ^{1}H NMR δ (500MHz, $CDCl_3$) : 2.86 (d,), 4.01 (m,), 5.3 (m,), 0.61-1.62 (s,). ^{13}C NMR δ (500MHz, $CDCl_3$) : 28.67, 26.19, 72.69, 34.63, 59.11, 55.69, 33.87, 30.96, 48.97, 38.86, 18.72,

Table I. ^{13}C-NMR and ^{1}H-NMR spectral values of 2-hydroxylstigmastane acetate compared with β-sitosterol acetate in literature recorded in $CDCl_3$ (500MHz)

Carbon no	Dept	^{13}C	Dept*	^{13}C *	^{1}H	^{1}H*
C-1	CH_2	39.73	CH_2	36.30		
C-2	CH	64.14	CH_2	27.50		
C-3	CH	70.77	CH	72.00	4.49	4.67(1H,m)
C-4	CH_2	27.92	CH_2	38.90		
C-5	CH	42.73	QC	138.84		
C-6	CH_2	28.20	CH	123.90		
C-7	CH_2	32.74	CH_2	32.20		
C-8	CH	35.54	CH	31.50		
C-9	CH	45.84	CH	50.30		
C-10	QC	30.17	QC	36.30		
C-11	CH_2	21.08	CH_2	21.20		
C-12	CH_2	39.10	CH_2	39.80		
C-13	QC	45.34	QC	42.60		
C-14	CH	56.13	CH	56.20		
C-15	CH_2	24.13	CH_2	24.40		
C-16	CH_2	29.70	CH_2	28.40		
C-17	CH	55.34	CH	56.10		
C-18	CH_3	12.19	CH_3	12.10		
C-19	CH_3	19.82	CH_3	19.30		
C-20	CH	38.07	CH_2	40.40		
C-21	CH_3	22.67	CH_3	21.50		
C-22	CH_2	34.75	CH_2	34.20		
C-23	CH_2	26.58	CH_2	26.20		
C-24	CH	36.18	CH	51.20		
C-25	QC	31.93	CH	30.00		
C-26	CH_3	18.60	CH_3	19.20		
C-27	CH_3	18.24	CH_3	21.20		
C-28	CH_2	25.04	CH_2	25.40		
C-29	CH_3	11.96	CH_3	12.20		
C-30	QC	173.78	QC	172.00		
C-31	CH_3	21.09	CH_3	21.20	2.2 (3H,s)	2.03 (3H, s)

* The chemical shift values (δ, ppm) were compared with what was obtained by Nur et al., 2017 previously. Assignments were made on the basis of COSY and HSQC correlations.

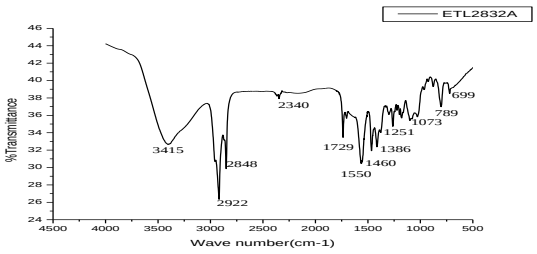
41.34, 47.91, 51.22, 24.03, 26.92, 60.39, 13.12, 15.32, 34.51, 17.20, 117.15, 124.41, 44.89, 34.98, 19.57, 19.89, 16.99, 173.06, 21.71.

Compound 3 (Rf value of 0.3) was also obtained as white powder. IR (cm^{-1} , KBr): 3400, 2938, 2863, 1655, 1535, 1460, 1237, 1027, 789. ^{1}H NMR δ (500MHz, $CDCl_3$) : 5.30 (d), 3.45 (m), 0.6-0.98 (s). ^{13}C NMR δ (500MHz, $CDCl_3$) : 37.29, 31.66, 72.01, 42.22, 140.96, 121.97, 31.90, 29.20, 56.06, 36.47, 23.04, 39.87, 57.02, 57.00, 24.31, 28.26, 49.96, 11.99, 19.85, 34.13, 18.80, 29.67, 26.09, 45.84, 21.08, 19.41, 19.06, 17.50, 13.73

DISCUSSION

Characterization of compound 1 (ETL128-32)

Compound 1 was obtained as a white powder with an R_f value of 0.4 ($CHCl_3$ 100%). The IR spectral (Supplementary 1) data showed an absorption band at 3415 cm^{-1} due to the presence



Supplementary 1. FTIR spectrum of compound 4 (2-hydroxystigmastane acetate)

of a hydroxyl group (O-H) band at 2922 – 2848 cm^{-1} due to the stretching vibration of -CH_3 and –CH_2. A strong absorption band at 1729 cm^{-1} revealed the presence of carbonyl (C=O) at 1389 cm^{-1}, indicating the presence of gem-dimethyl of the form –CH-$(CH_3)_2$, at 125 cm^{-1} and 1073 cm^{-1} are due to C-O stretching vibration. Band at 1460 showed the

Table II. ^{13}C-NMR and ^{1}H-NMR spectral values of Ergost-5,6-epoxy-22-en-3-yl- acetate compared with literature recorded in CDCl3 (500MHz)

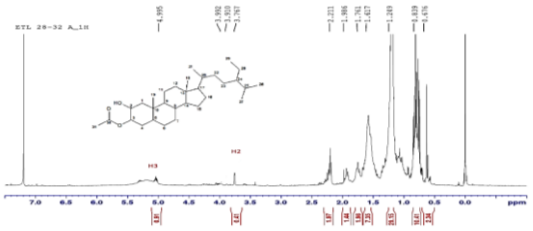
Carbon no	Dept	^{13}C	^{13}C *	^{1}H	^{1}H*
C-1	CH_2	28.67	29.74		
C-2	CH	26.19	28.32		
C-3	CH	72.69	71.34	4.01(d,1H)	3.09(d,0.95H)
C-4	CH_2	35.63	39.72		
C-5	QC	59.11	62.52		
C-6	CH	55.70	56.27	2.86 (d)	2.43(d)
C-7	CH_2	33.87	33.35		
C-8	CH	30.96	32.43		
C-9	CH	48.97	51.02		
C-10	QC	38.86	38.01		
C-11	CH_2	18.72	21.34		
C-12	CH_2	41.34	38.32		
C-13	QC	47.91	42.18		
C-14	CH	51.22	56.02		
C-15	CH_2	24.03	24.15		
C-16	CH_2	26.92	27.20		
C-17	CH	60.39	63.54		
C-18	CH_3	13.12	17.06		
C-19	CH_3	15.32	12.06		
C-20	CH	34.51	34.48		
C-21	CH_3	17.20	18.63		
C-22	CH_2	117.15	131.22	5.3(dd, 2H)	5.02(dd,2H)
C-23	CH_2	124.41	135.13		
C-24	CH	44.89	35.04		
C-25	CH	34.89	36.68		
C-26	CH_3	19.57	20.55		
C-27	CH_3	19.89	20.36		
C-28	CH_3	16.99	19.94		
C-29	CO	173.07	170.56		
C-30	CH_3	21.71	21.92		

* The chemical shift values (δ, ppm) were compared with what was obtained by Hang and Dussault, 2010 previously. Assignments were made on the basis of COSY and HSQC correlations.

presence of cyclic methylene group (-CH_2).

The ^{1}H- NMR spectrum (Supplementary 2) of compound 1 showed signals between 0.67-3.99 ppm. The ^{1}HNMR integration showed the presence of 54 protons. The spectrum revealed seven methyl protons at δ_H 0.67, 0.70, 0.83, 0.85, 1.15, 1.20, and 2.20, corresponding to H18, H27, H26, H29, H21, H19, and H31, respectively. The methyl protons at (H-31) resonated at δ_H2.20. These are methyl protons from the acetate group. The proton at δ_H 3.75 is the proton H2, which is directly attached to the carbon that bears the hydroxyl group. At δ_H 4.49 is the proton attached to the C-O carbon of the acetate H-3.

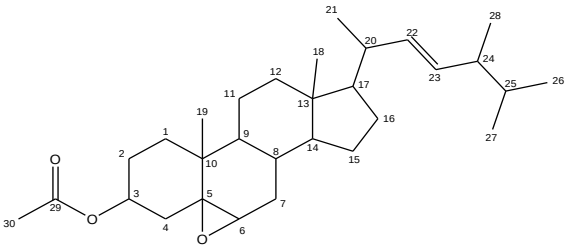
^{13}C-NMR spectrum (Supplemetary 3) revealed 31 carbon atoms in compound 1, as shown in Table I. The important ^{13}C signals are the signals at δ_C 173.78 (C-30), which is the carbonyl carbon (C=O) of acetate, δ_C 70.77 at(C-3) for C-O



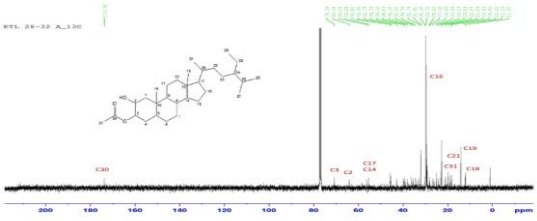
Supplementary 2. ^{1}H-NMR spectrum of compound 4 (2-hydroxystigmastane acetate)

and signal at δ_C 64.14 for (C-2) that bears the hydroxyl group.

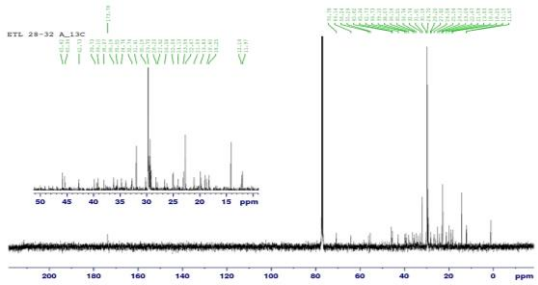
The DEPT-135 spectrum (Supplementary 4) revealed seven methyl, ten methine, eleven methylene, and three quaternary carbons. The spectrum showed a total of twenty-eight signals on both sides.



[2]



Supplementary 3. ^{13}C-NMR spectrum of compound 4 (2-hydroxystigmastane acetate)



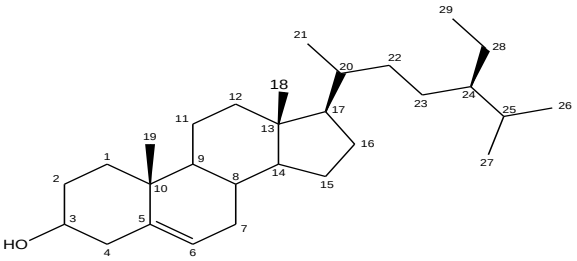
Supplementary 4. Expanded ^{13}C-NMR spectrum of compound 4 (2-hydroxystigmastane acetate)

COSY spectrum (Supplementary 5) for the isolated compound 1 showed the correlation between protons as follows: H2-H1, H3-H5, and H3-H4. HSQC spectrum (Supplementary 6) also supported the structural elucidation of compound 1 by showing the correlation between carbons and their attached protons. H18 correlated with C18 at δc 12.19, H19 correlated with C19 at δc 19.82, H4 correlated with C4 δc 27.92, H2-C2 at δc 64.14 and H3-C3 at δc 70.77. COSY and HSQC were used for the assignments of carbons and protons. All the spectral data of this compound are in excellent agreement with that of β-sitosterol acetate earlier reported (Nur et al., 2017). The only difference is that there is no double bond in compound 1 and also the presence of hydroxyl group on carbon 2 of the isolated compound, which is absent in β-sitosterol acetate. With all the spectral data and comparison with closely related compounds from the literature (Nur et al., 2017), compound 1 is proposed to be 2-hydroxylstigmastane acetate with molecular formula $C_{31}H_{54}O_3$.

Characterization of compound 2 (ETL2$_9$)

Compound 2 R_f value of 0.6 $CHCl_3$ 100%) was obtained as a white powder. A single spot was observed under the UV lamp. The IR spectrum (Supplementary 7) showed the prominent functional group at 1729 cm^{-1}, which indicates the presence of carbonyl functional group (C=O), 1565 cm^{-1} shows the presence of double bond (C=C), band at 1460 cm^{-1} is for C-O bond, 3415 cm^{-1} represent the presence of O-H group which may be due to the water introduced during sample preparation.

^{1}H- NMR (Supplementary 8) showed the steroidal nucleus with the δ_H 2.86 (d, H-6), δ_H4.01 (m, H-3) and δ_H5.30 (dd, H-22 and H-23). ^{1}H-NMR spectrum integration revealed forty-eight (48) protons with seven methyl groups.

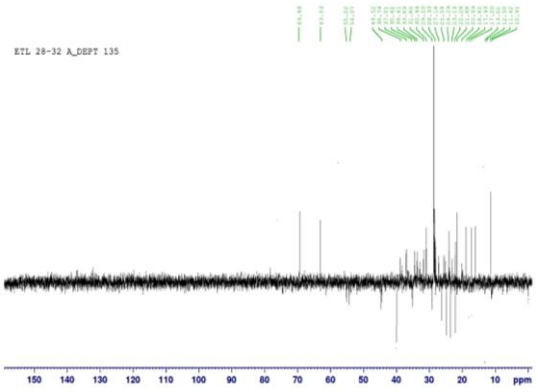


[3]

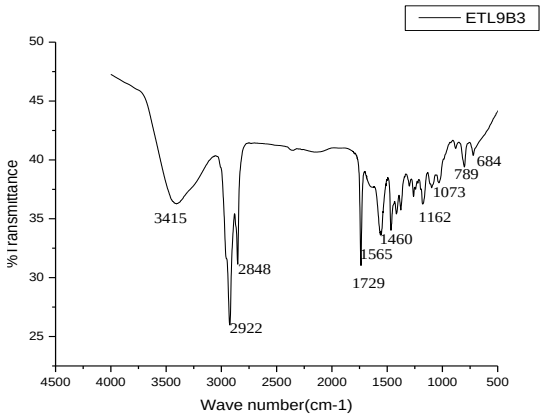
^{13}C-NMR spectrum (Supplementary 9) showed a total of thirty carbon atoms. In contrast, the DEPT-135 spectrum (Supplementary 10) revealed the different types of carbon atoms present as follows: seven methyl (-CH_3), eleven methines (-CH), eight methylene (-CH_2) and four quaternary carbons. These quaternary carbons were not shown on the spectrum. The major ^{13}C signals, as shown in Table II, are the signal at δ_C173.07 which shows the presence of carbonyl carbon, 117.15 and 124.41 due to C-22 and C-23, respectively, and the signal at 72.69 corresponds to the oxymethine carbon (C-3). Comparing all the spectral data obtained with the literature (Hang and Dussault, 2010), compound 2 is proposed to be Ergost-5, 6-epoxy-22-en-3-yl-acetate with molecular formula $C_{30}H_{48}O_3$.

Characterization of compound 3 (ETL3$_6$ G)

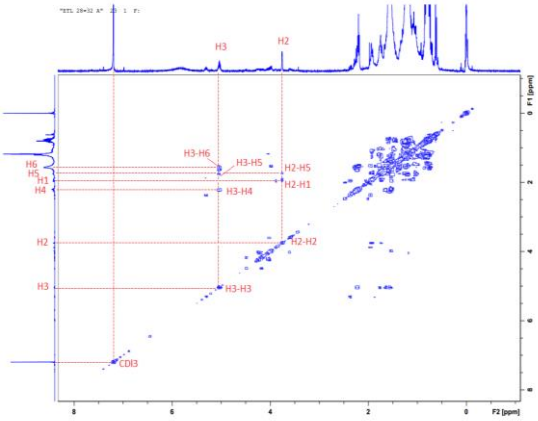
Compound 3 from ethanol leave extract (R_f value of 0.3 Hex/$CHCl_3$ 3:7) was also obtained as a white powder. All the spectral data were similar to that of β-sitosterol previously characterized and reported on ethyl acetate stem bark extract of *N. pobegunii* (Adepoju et al., 2022; Ododo et al., 2016). Therefore, compound 3 is confirmed to be β-sitosterol from ethanol extract of leaves of *N. pobegunii*. The ^{13}C-NMR data and important ^{1}H-NMR spectra are shown in Table III.



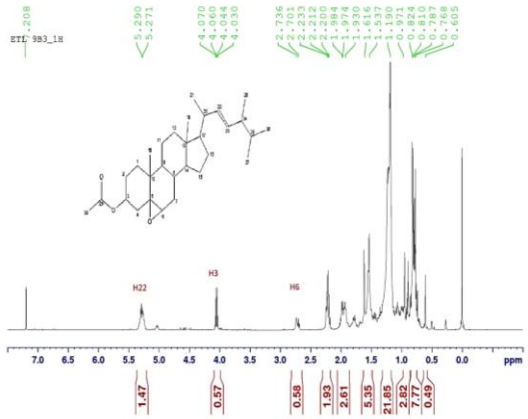
Supplementary 5. DEPT spectrum of compound 4 (2-hydroxystigmastane acetate)



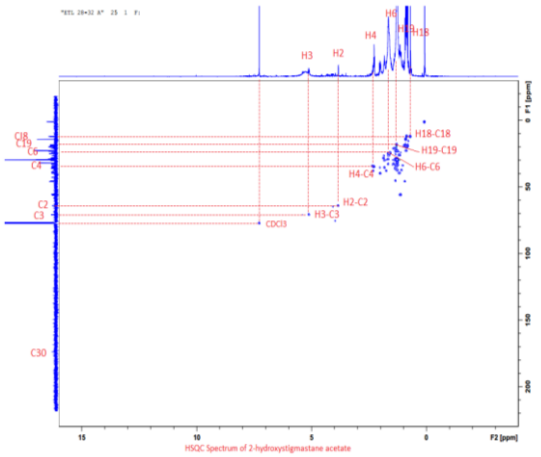
Supplementary 8. FTIR spectrum of compound 5 (Ergost-5,6-epoxy-22-en-3yl acetate)



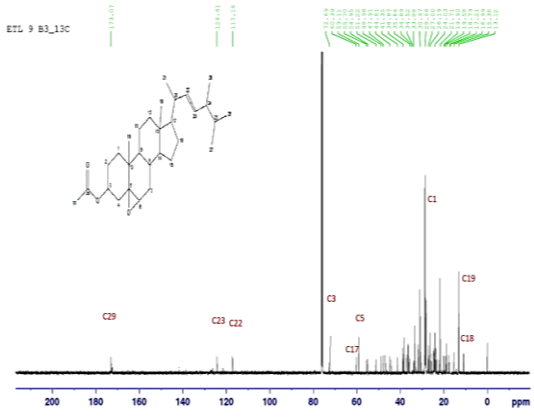
Supplementary 6. COSY spectrum of compound 4 (2-hydroxystigmastane acetate)



Supplementary 9. ^{1}H-NMR spectrum of compound 5 (Ergost-5, 6-epoxy-22-en-3yl acetate)



Supplementary 7. HSQC spectrum of compound 4 (2-hydroxystigmastane acetate)



Supplementary 10. ^{13}C-NMR spectrum of compound 5 (Ergost-5, 6-epoxy-22-en-3yl acetate)

Table III: 13Cand 1H chemical shift values for β-sitosterol from leaves extract of N. pobeguinii recorded in CDCl3 (500MHz).

Carbon no	Dept	^{13}C	Dept*	^{1}H	^{1}H*
C-1	CH_2	37.29	37.28		
C-2	CH_2	31.66	31.69		
C-3	CH(OH)	72.01	71.82	3.48(m)	3.54(m)
C-4	CH_2	42.22	42.33		
C-5	QC(=)	140.96	140.77	-	-
C-6	CH(=)	121.97	121.73	5.30(t)	5.37(overlapping,t)
C-7	CH_2	31.90	31.93		
C-8	CH	29.20	31.93		
C-9	CH	56.06	50.16		
C-10	QC	36.47	36.51		
C-11	CH_2	23.04	21.11		
C-12	CH_2	39.87	39.80		
C-13	QC	57.13	42.34		
C-14	CH	57.00	56.79		
C-15	CH_2	24.31	24.33		
C-16	CH_2	28.26	28.27		
C-17	CH	49.96	56.08		
C-18	CH_3	11.99	11.89	0.60(s)	0.7(s)
C-19	CH_3	19.85	19.42	0.94(s)	1.03(s)
C-20	CH	34.13	36.17		
C-21	CH_3	18.82	18.84		
C-22	CH_2	29.67	33.98		
C-23	CH_3	26.09	26.11		
C-24	CH	45.84	45.86		
C-25	CH	21.08	29.19		
C-26	CH_3	19.41	19.84		
C-27	CH_3	19.06	19.06		
C-28	CH_2	17.50	21.10		
C-29	CH_3	13.73	12.01		

* The chemical shift values (δ, ppm) were compared with what was obtained by Adepojuet al., 2022; Ododo et al., 2016 previously. Assignments were made on the basis of COSY and HSQC correlations.

CONCLUSION

The established chemical and spectral evidence and comparison of the present results with the previous data, the isolated compounds are confirmed to be 2-hydroxylstigmastane acetate (1), Ergost-5, 6-epoxy-22-en-3-yl-acetate (2) and β-sitosterol (3). Compounds 1 and 2 are being reported for the first time in *N. pobeguinii* to the best of our knowledge

ACKNOWLEDGEMENTS

The authors acknowledge the financial support of the Tertiary Education Trust Funds (TETFund) of Nigeria and facilities offered by the School of Chemical Sciences, Universiti Sains Malaysia (USM), Malaysia to AAJ.

REFERENCES

Adepoju, A. J., Falade, A. V., Ayodele, E. T., Oladoye, S. O., Lim, G. K., OO, C. W., et al. (2020). Phytochemical Screening, Antibacterial and antioxidant potential of the stem bark extracts of Nauclea pobeguinii. *Sci. Focus, 25*, 38–45.

Adepoju, A. J., Oladoye, S, O., Ayodele, E. T., Falade, V. A., Lim, G. K and OO, C. W.(2022). Isolation and Identification of β-sitosterol, 7-hydroxystigmast-22-en-3,6-dione and 3β, 24(S)-dihydroxycholesta-5, 25-diene-7-one fromstem bark of *Nauclea pobeguinii.Traditional Medicine Journal.*Vol. 27(3), p 210-217.https://DOI: 10.22146/mot.77324

Ambe, A.S., Ouattara, D., Tiebre, M.-S., Ta, B., Vroh, G.N.Z., N'Guessan, K.E. (2015). Diversité des plantes médicinales utilisées dans le traitement traditionnel de ladiarrhée sur les marchés d'Abidjan (Côte d'Ivoire). *Journal of Animal and Plant Sciences*. 26, 4081 – 4096.

Bett, J. L. (2002). Medicinal plants sold in Yaoundé markets, Cameroon. *African Study Monographs, 23*, 47–64.

Das, K., Tiwari, R. K. S., Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agents. Current methods and future trends. *Journal of Medicinal Plant Research*. 4, 104 - 111.

Hang, J., Dussault, P. (2010). A concise synthesis of β-sitosterol and other phytosterols. *Steroids*, 75, 879 - 88.

Haudecoeu, R., Marin, P., Basile, P., Maxime, R., Germain, S. T., Ahcene, B., et al. (2018). Traditional uses, phytochemistry and pharmacological properties of African Nauclea species. *A review. J. of Ethnopharmacol, 212*, 106 - 136.

Hesham, H. A., Nour, A. H., Yunus, R. M. (2016). Techniques for extraction of essential oils from plant: A review. *Australian Journal of Basic and Applied Sciences*. 10, 117-127.

Islam, S., & Lucky, R. (2019). A Study on different plants of the Apocynaceae family and their medicinal uses. *Univers J Pharm Res, 4* (1), 42-6.

Luzakibanza, M. M. (2012). *Etude phytochimique et pharmacologique de plantes antipaludiques utilisées en médecine traditionnelle Congolaise. Congo D. R., editor. Belgium: Université de Liège & Université de Kinshasa.* Congo D. R., editor. Congo D. R., editor.

MacDonald, I., Olorunfemi, D.I. (2005). Plants used for medicinal purposes by the Koma people of Adamawa State, Nigeria. *Indigenous Knowledge and Development Monit*or, 8, 18 - 25.

Mbiantcha, M., Tsafack, E. G., Ateufack, G., & al., e. (2018). Analgesic, antiinflammatory and anti-arthritic properties of aqueous and methanolic stem bark extracts from Nauclea pobeguinii (Rubiacee) in rats. *Journal of Complementary and Integrative Medicine, 170*, 20.

Mesia, G. K., Tona, G. L., Penge, O., & al, e. (2005). Antimalarial activities and toxicities of three plants used as traditional remedies for malaria in the Democratic Republic of Congo:Croton mubango, Nauclea pobeguiniiandPyrenacantha staudtii. *Annals of Tropical Medicine & Parasitology* , 99.

Njoya, E. M., Aristide, M. M., Pierra, M., Augustin, E. N., & yndy, J. M. (2017). Phytochemical analysis with free radical scavenging, nitric oxide inhibition, and antiproliferative activity of Sarcocephalus pobeguiniiextracts. *BMC Complementary and Alternative Med, 17*, 199 – 215.

Nur, S. A., Wan, Y.W.A, Noor, A. S. (2017). The chemical constituents from young tubers of *Hydrophytum formicarum*. *Malaysian Journal of Analytical Sciences*, 21, 291 - 297.

Ododo, M. M. Manash, K. C., Ahmed, H. D. (2016). Structure Elucidation of β-sitosterol with antibacterial activity from the root bark of *Malva parviflora*. *Springer plus*; 5; 1210.

Ribeiro, D. A., Damasceno, S. S., Boligon, A. A., de Menezes IRA, d. A., & da Costa, J. G. (2017). Chemical profile and antimicrobial activity of Secondatia floribunda A. DC (Apocynaceae). *Asian Pac J Trop Biomed, 7* (8), 739.

Samoisy, A. K., & Mahomoodally, M. F. (2015). Ethnopharmacological analysis of medicinal plants used against noncommunicable diseases in Rodrigues Island, Indian Ocean. *J Ethnopharmacol* , 20-38.

Xu, B., Descalzi, G., Ye, H. R., Zhuo, M., & Wang, Y. W. (2012). Translational investigation and treatment of neuropathic pain. *Journal of Molecular Pain, 8*, 15.