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Glycosylated stigmasterol from the rind of *Napoleonaea imperialis*

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Abstract. *Napoleonaea imperialis* rinds were separated from the seeds and extracted via maceration using methanol. The crude methanol extract was partitioned with *n*-hexane and dichloromethane to yield *n*-hexane fraction (11.01 g), dichloromethane fraction (35.16 g) and methanol fraction (101.75 g). Dichloromethane fraction was chromatographed and purified to give a compound whose structure was elucidated using one-dimension and two-dimension nuclear magnetic resonance (NMR) experiments. The isolated compound was characterized as glycosylated stigmasterol.

***Keywords*:** *Napoleonaea imperialis*; rind; chromatography; saponins; glycosylated stigmasterol.

1. Introduction

Natural products have been the source of most active ingredients of medicine, innovative therapeutic agents for infectious diseases (both bacterial and fungal), cancer, lipid disorders and immunomodulation, and provide starting point for new synthetic compounds with diverse structures and multiple stereocenters [1-3]. The oldest phytochemical-based drugs are analgesics which contain salicin [4], opium with a potent narcotic component (morphine) and taxol a chemotherapeutic drug isolated from *Papaver somniferum* and *Taxus brevifolia* respectively [4-6]. *Napoleonaea imperialis* P. Beauv. (Family Lecythidaceae), commonly called Napoleon's Hat is known as "ukpodu" or "ukpakonrisa" in southern Nigeria and predominantly found in bush fallows and secondary bushes [7]. The bark and fruit rind are used to treat respiratory tract infections while the twigs are used as traditional chew stick for oral hygiene [8, 9]. A molluscicidal triterpenoid saponin (Napoleonaside A) and a novel bioactive triterpenoid saponin (Napoleonaside B) have been isolated from the seed of *N. imperialis* [10, 11]. Two new oleanane-type glycosides, Napoleonaside G and Napoleonaside R, and other compounds of pharmaceutical interest have also been found in the rind extract of *N. imperialis*; while neral and geranial are major compounds reported to be present in the essential oil of *N. imperialis* rind and are responsible for the characteristic aroma of the oil [12, 13]. This paper reports the isolation and structural elucidation of glycosylated stigmasterol from the extract of *Napoleonaea imperialis* rind.

2. Experimental

2.1. Materials

Chemicals used for this work include deuterated dimethylsulphoxide (DMSO-d_6), acetic anhydride and sulphuric acid (all products of Sigma-Aldrich). All solvents used were redistilled. Analytical thin layer chromatography (TLC) was conducted on silica gel (Merck F_{253}) precoated aluminum plates. Vacuum liquid chromatography and column chromatography were carried out using silica gel 70-230 and 60-200 mesh ASTM (60H Merck) respectively.

2.2. TLC spots detection

One dimension TLC was done by spotting the isolate on a 5x5 cm TLC plate and developed. Detection of spots on developed TLC plate was done under UV light at wavelength of 254 nm and further detection was done using spray reagent (10 % sulphuric acid in methanol) followed by heating at 105 ℃ for 2-5 minutes.

2.3. Extraction and partitioning

Fresh fruits of *N. imperialis* were harvested from Owerri, Imo state, Nigeria. The rinds were separated from their seeds, chopped, air-dried, and pulverized at room temperature (25 ℃), and subsequently weighed. Methanol (1.4 L) was used to macerate the pulverized rind (617.2 g) for 48 hours. The resulting extract was concentrated to dryness using a rotary evaporator (Labrota 4002) at 40 ℃ to afford a brown sticky crude extract (148.12 g). The crude extract was dissolved in 200 ml methanol and subsequently partitioned successively between 400 ml *n*-hexane and 400 ml dichloromethane in a separatory funnel. Each of the partitioned fraction was concentrated to yield *n*-hexane fraction (11.01 g), dichloromethane fraction (35.16 g) and methanol fraction (101.75 g) which were preserved for further analysis.

2.4. Chromatography

Vacuum liquid chromatography method was used for this separation [14]. Briefly, 32.97 g of dichloromethane fraction and 20 g of silica gel were properly mixed to obtain a light brown powder. The mixture was poured into a sintered glass funnel (9.8 cm x 10.4 cm) that was already packed with 130 g of silica gel (4.5 cm) and was evenly distributed to a height of 6 cm. Gradient elution using different binary solvent systems of *n*-hexane (*n*-Hex), ethyl acetate (EtOAc) and methanol (MeOH) were carried out and fractions of 200 ml each collected. A total of 25 fractions were obtained and combined

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based on TLC analysis to give 13 major fractions (F_1 - F_{13}). The fractions were subsequently dried at 25 ℃, weighed and stored in glass vials.

2.5. Purification

F_9 (light brown powder) which eluted with EtOAc-MeOH (9.5:0.5) mobile phase from VLC weighed 1.9 g. Column chromatography was employed in the purification of F_9. F_9 was chromatographed over 60 g of silica gel packed in a glass column (91 cm x 2.5 cm) and eluted with EtOAc - *n*-Hex (6:4), EtOAc - *n*-Hex (8:2), 100% EtOAc and EtOAc - MeOH (9.5:0.5). A total of 75 fractions of 20 ml each were collected. Fractions 11-38 (which eluted with EtOAc - *n*-Hex, 8:2) were combined to give 500 mg of a white amorphous compound which precipitated out from the solution. TLC analysis using 100% EtOAc as mobile phase, showed that the compound was not visible under the UV light but gave a purple spot with R_f value 0.4 after the developed TLC plate was treated with spray reagent and heated.

2.6. Confirmatory test

Freshly prepared Liebermann-Burchard reagent (1 ml) was slowly added to 5 mg of the isolated compound dissolved in 1 ml dichloromethane [14]. A green colouration was observed.

2.7. NMR spectroscopy

All NMR data were acquired at 25 ℃ on a Bruker 600 MHz spectrometer (^{1}H, 600.06 MHz) equipped with a 5 mm cryoprobe. The sample (~ 6 mg) was solubilized in 550 µL DMSO-d_6 solution after briefly heating at 45 ℃ and mixing. The solution was then transferred to a 5 mm NMR tube. ^{1}H {^{13}C} NMR experiment was acquired with spectral width of 9090.9 Hz, 32768 data points, 8 scans and 1.5 s relaxation delay. ^{13}C NMR experiments was acquired with spectral width of 35714.3 Hz, 32768 data points, 3840 scans and 1.5 s relaxation delay. 2D NMR data were acquired with ^{1}H spectral widths of 4424.8 Hz (COSY, TOCSY and NOESY), ^{1}H/^{13}C spectral widths of 4424.8 Hz / 22123.9 Hz (HSQC and HSQC-TOCSY) and ^{1}H/^{13}C spectral widths of 4424.8 Hz / 22123.9 Hz (HMBC). COSY data was acquired with 2 scans and 1536 * 768 (t1 * t2) points, TOCSY data was acquired with 8 scans and 1024 * 384 (t1 * t2) points, NOESY data was acquired with 20 scans and 1280 * 512 (t1 * t2) points, HSQC data was acquired with 8 scans and 1024 * 768 (t1 * t2) points, HSQC-TOCSY data was acquired with 16 scans and 871 * 768 (t1 * t2) points and HMBC data was acquired with 44 scans and 1536 * 256 (t1 * t2) points. Chemical shifts were referenced to the residual DMSO signal at 2.50 ppm (^{1}H) and 39.5 ppm (^{13}C). All spectra were processed and analyzed with MestreNova (version 14.0.1-23559).

3. Results and discussion

The green colour observed when fresh Liebermann-Burchard reagent was added to the compound indicated the presence of a steroidal skeleton [15-17] and the purple spot observed after treatment with the spray reagent and heating also confirmed the presence of steroidal sapogenin [18]. The isolate's invisibility under the UV light is a strong indication that there is absence of conjugated double bonds in its structure. Structure of the isolate (Figure 1) was determined using ^{1}HNMR and ^{13}CNMR experiments (Figures 2 and 3) aided by connectivities observed in HSQC, HMBC, COSY, HSQC-TOCSY and NOESY experiments (Table 1).

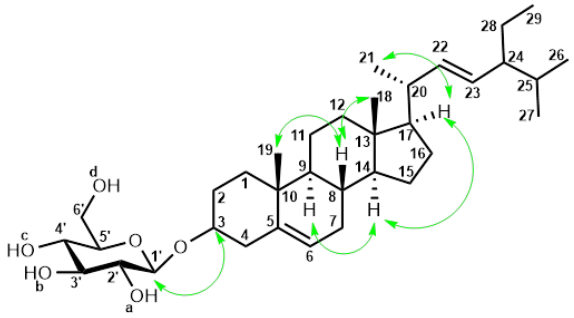

Figure 1. Structure and numbering of glycosylated stigmasterol used in this report. The sugar moiety was identified as β-glucose from TOCSY spin-lock pattern and ^{1}H/^{13}C NMR chemical shifts. NOESY correlations that establish stereochemistry is indicated with green arrows. The stereochemistry at C-3 and C-24 could not be established with the current dataset.

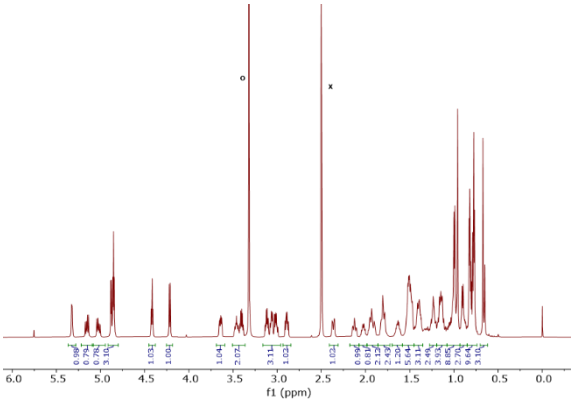

Figure 2. ^{1}H NMR spectrum of glycosylated stigmasterol acquired in DMSO-d_6. Signal from solvent moisture (water) is marked with 'o' while DMSO residual solvent signal is marked with 'x'.

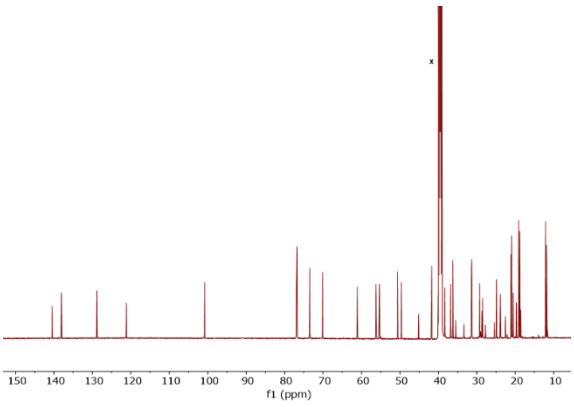

Figure 3. ^{13}C NMR spectrum of glycosylated stigmasterol acquired in DMSO-d_6. Signal from DMSO is marked with 'x'. Minor peaks are impurity signals of a glycosylated molecule not assigned.

Table 1. ^{13}C and ^{1}H chemical shift data of glycosylated stigmasterol in DMSO-d_6

Aglycone			Sugar moiety		
Position	**δ ^{13}C**	**δ ^{1}H**	**Position**	**δ ^{13}C**	**δ ^{1}H**
1	36.8	1.79 (1H, m), 0.99 (1H, m)	1'	101.8	4.22 (1H, d, 7.8 Hz)
2	29.3	1.81 (1H, m), 1.48(1H, m)	2'	73.5	2.89 (1H, ddd, 8.9 Hz, 7.8 Hz, 4.8 Hz)
3	76.9	3.46 (1H, tt, 11.3 Hz, 4.2 Hz)	3'	76.8	3.12 (1H, td, 8.8 Hz, 4.8 Hz)
4	38.3	2.36 (1H, m), 2.12 (1H, m)	4'	70.1	3.01 (1H, td, 9.1 Hz, 5.1 Hz)
5	140.5	-	5'	76.8	3.06 (1H, ddd, 9.9 Hz, 5.9 Hz, 2.1 Hz)
6	121.2	5.32 (1H, m)	6'	61.1	3.64 (1H, td, 11.7 Hz, 5.9 Hz), 3.40 (1H, ddd, 11.5 Hz, 5.7 Hz, 2.0 Hz)
7	31.4	1.92 (1H, s), 1.49 (1H, m)	^{a}O		4.86 (1H, d, 5.2 Hz)
8	31.3	1.51 (1H, m)	^{b}O		4.88 (1H, d, 4.8 Hz)
9	49.6	0.89 (1H, m)	^{c}O		4.85 (1H, d, 5.4 Hz)
10	36.2	-	^{d}O		4.41 (1H, t, 5.8 Hz)
11	20.6	1.49 (1H, m), 1.40 (1H, m)			
12	39.1	1.94 (1H, m) 1.15 (1H, m)			
13	41.7	-			
14	56.3	0.99 (1H, m)			
15	23.9	1.52 (1H, m), 1.02 (1H, m)			
16	28.5	1.64 (1H, m), 1.23 (1H, m)			
17	55.3	1.13 (1H, m)			
18	11.9	0.67 (3H, s)			
19	19.1	0.96 (3H, s)			
20	39.9	2.02 (1H, m)			
21	21.1	0.99 (3H, d, 6.6 Hz))			
22	138.0	5.15 (1H, dd, 15.2 Hz, 8.7 Hz)			
23	128.8	5.02 (1H, dd, 15.2 Hz, 8.7 Hz)			
24	50.6	1.52 (1H, m)			
25	31.4	1.39 (1H, m)			
26	18.9	0.77 (3H, d*)			
27	20.9	0.82 (3H, d*)			
28	24.9	1.39 (1H, m), 1.13 (1H, m)			
29	12.1	0.77 (3H, m*)			

* Overlapping signals

Thirty-five carbons were identified from ^{13}C NMR and HSQC spectra (Table 1); these include one glucose anomeric carbon signal at δ101.8 (C-1') and five ring residue signals, δ73.5 (C-2'), δ76.8 (C-3'), δ70.1 (C-4'), δ76.8 (C-5'), δ61.1 (C-6') belonging to the sugar moiety. The glucose residue was identified from the corresponding anomeric proton TOCSY spinlock pattern while the glycosyl linkage configuration was determined as beta (β) based on the ^{1}H chemical shift at 4.22 ppm (Figure 1). ^{1}H NMR and ^{13}C NMR assignments, aided by heteronuclear single quantum coherence (HSQC) spectrum (Figure 4) and verified with other two dimension (2D) spectra (Figures 5 and 6), show that the isolate (Figure 1) has one unsaturated and two saturated quaternary carbons, δ140.5 (C-5), δ36.2 (C-10) and δ41.7 (C-13); six primary carbons (methyl groups), δ11.9 (C-18), δ19.1 (C-19), δ21.1 (C-21), δ18.9 (C-26), δ20.9 (C-27) and δ0.77 (C-29); ten secondary carbons (methylenes), δ36.8 (C-1), δ29.3 (C-2), δ76.9 (C-3), δ38.3 (C-4), δ31.4 (C-7), δ20.6 (C-11), δ39.1 (C-12), δ23.9 (C-15), δ28.5 (C-16) and δ24.9 (C-28); and ten tertiary carbons, δ121.2 (C-6), δ31.3 (C-8), δ49.6 (C-9), δ56.3 (C-14), δ55.3 (C-17), δ39.9 (C-20), δ138.0 (C-22), δ128.8 (C-23), δ50.6 (C-24) and δ31.4 (C-25) assigned to the aglycone. Protons at δ4.86 (H-^{a}O), 4.88 (H-^{b}O), 4.85 (H-^{c}O), 4.41 (H-^{d}O) that are not attached to carbons in the HSQC spectrum were assigned as the sugar hydroxy protons.

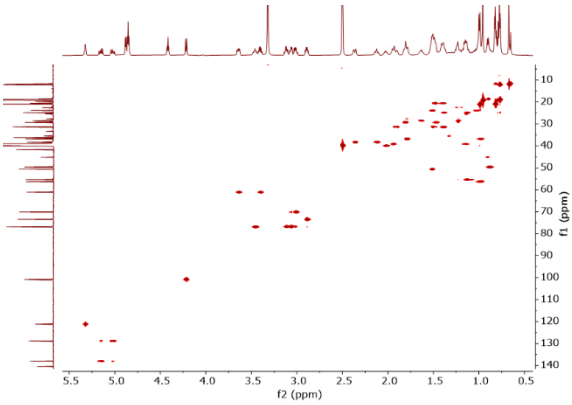

Figure 4. HSQC NMR spectrum (non-edited version) of glycosylated stigmasterol acquired in DMSO-d_6.

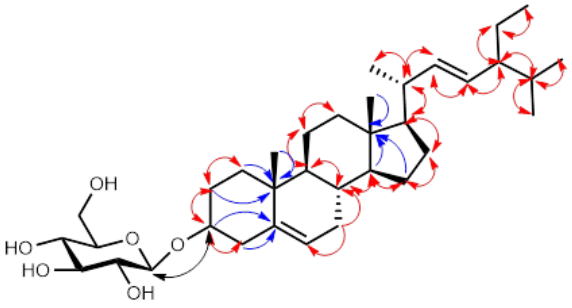

Figure 5. Glycosylated stigmasterol showing COSY and HSQC-TOCSY connectivity with red arrows. Key HMBC connectivity to quaternary carbons are shown with blue arrows.

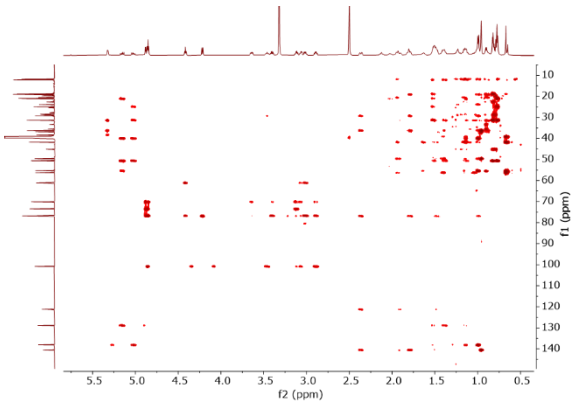

Figure 6. HMBC NMR spectrum of glycosylated stigmasterol acquired in DMSO-d_6.

Cross peaks from NOESY (Figure 1) show that the anomeric proton δ4.22 (H-1') correlated with δ3.46 (H-3) of the aglycone; proton at δ4.86 (H-^{a}O) correlated with δ4.22 (H-1), δ3.06 (H-5') and δ4.41 (H-^{d}O); δ4.88 (H-^{b}O) correlated with δ4.22 (H-1") and δ3.12 (H-3') while δ4.85 (H-^{c}O) correlated with δ3.01 (H-4'), δ4.41 (^{d}O) and δ2.89 (H-2"); δ4.41 (H-^{d}O) correlated with δ4.86 (H-^{a}O), δ4.85 (H-^{c}O), δ3.64 (H-6') and δ3.06 (H-5'). HSQC-TOCSY (Figure 5) shows that carbon δ101.8 (C-1') directly correlated with δ4.22 (H-1') of the sugar moiety is correlated with δ3.46 (H-3) which is also directly correlated with δ76.9 (C-3) of the aglycone. Protons at δ4.86 (H-^{a}O), δ4.88 (H-^{b}O), δ4.85 (H-^{c}O), δ4.41 (H-^{d}O) showed no correlation in HSQC-TOCSY.

Two olefinic carbon signals at δ140.5 and δ121.2 were assigned to C-5 and C-6, while another pair of olefinic carbons δ138.0 and δ128.8 were designated C-22 and C-23, respectively, based on connectivity from neighbouring signals in the 2D NMR data. C-6, C-22 and C-23 are trisubstituted olefinic linkages. These peaks are reported as the striking characteristics of stigmasterol [17, 19-22]. Connectivities from long range HMBC (Figures 5 and 6) show that the olefinic carbons δ140.5 (C-5) and δ121.2 (C-6) are correlated with δ2.36 (H-4) and δ1.49 (H-7) respectively, while the second pair of olefinic carbons δ138.0 (C-22) and δ128.8 (C-23) are correlated with δ2.02 (H-20) and δ1.52 (H-24). The large coupling of 15.2 Hz between H_{22} and H_{23} (Table 1) is indicative of protons in anti-parallel orientation as is the case for the E-isomer of the double bond shown (Figures 1 and 5). HMBC also shows correlation between δ101.8 (C-1') and δ3.46 (H-3) which confirmed that the sugar moiety is attached to C-3 position of the aglycone. COSY (Figure 5) shows that the isopropyl group, δ31.4 (C-25), δ18.9 (C-26) and δ20.9 (C-27) correlated with δ1.52 (H-24) and δ1.13 (H-28). Correlations observed in the 2D experiments were similar to those reported for stigmasterol isolated from *Heliotropium indicum* Linn [23]. From 2D experiments, all proton signals belonging to the sugar moiety were assigned and the position of attachment to C-3 of the aglycone was also confirmed. The sugar moiety carbons were similar to that of the steroidal saponin isolated from *Dioscorea panthaica* [24]. All range correlations observed were consistent with the structure in Figure 1. The isolate was confirmed as glycosylated stigmasterol (Figure 1) after comparison with literature [19, 20, 25].

A review on the pharmacological studies of stigmasterols shows their anti-osteoarthritic, anti-hypercholestrolemic, cytotoxicity, anti-tumor, antimutagenic, antioxidant, hypoglycemic, antifungal activities, and analgesic potential [26-30]. Steroidal saponins are generally reported to have anthelmintic, immunomodulatory, anti-inflammatory, anti-melanogenic and anti-thrombotic activities [31-35].

4. Conclusions

Napoleonaea imperialis rind is a rich source of glycosylated stigmasterol, a pharmacologically useful compound. Isolation and characterization of glycosylated stigmasterol were successfully achieved using various chromatographic methods and NMR experiments.

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Conflict of interest

Authors declare no conflict of interest.

References

[1]. L.A. Harvey, Natural products in drug discovery, Drug Discovery Today 13 (2008) 894-901.

[2]. K. Altmann, The merger of natural products synthesis and medicinal chemistry: on the chemistry and chemical biology of epothilones, Organic and Biomolecular Chemistry 2 (2004) 2137-2152.

[3]. E.F. Koehn, T. G. Carter, The evolving role of natural products in drug discovery, Nature Reviews 4 (2005) 206-220.

[4]. T. Hohlfeld, N. Zimmermann, A. Weber, G. Jessen, H. Weber, K. Schror, H.D. Holtje, R. Ebel, Pyrazolinone analgesics prevent the antiplatelet effect of aspirin and preserve human platelet thromboxane synthesis, Journal of Thrombosis and Haemostasis 6 (2008) 166-173.

[5]. I. Bulduk, F. Taktak, Isolation and characterization of antitumor alkaloid from poppy capsules (*Papver somniferum*), Journal of Chemistry 1 (2013) 1-4.

[6]. M.J. Moshi, Current and future prospects of integrating traditional and alternative medicine in the management of diseases in Tanzania, Tanzania Journal of Health Research 7 (2005) 159-167.

[7]. O. James, E.A. Friday, E.G. Unekwuojo, Antihypertensive effect of methanol extract of *Napoleonaea imperialis* (P. Beauv) in adrenaline induced hypertensive albino rats, International Journal of Biochemistry Research and Review 1 (2011) 47-57.

[8]. G.I. Ndukwe, C. M. Ojinnaka, A.O. Oyedeji, N. Nxasana, T. Apalata, Antibacterial activity of the fruit of *Napoleoneae imperialis*, Journal of Innovation and Research Health Science and Biotechnology 1 (2015) 1-11.

[9]. M. Idu, A. Odaro, T. Umweni, L. Ojelede, Ethnobotanical plants used for oral healthcare among the Esan tribe of Edo state, Nigeria, Ethnobotanical Leaflets 13 (2009) 548-563.

[10]. C.M. Ojinnaka, D.C. Okpala, A Molluscicidal triterpenoid saponin from the fruits of *Napoleonaea imperialis* P. Beauv. (Lecythidacaea), Journal of Applied Science and Environmental Management 16 (2012) 213-216.

[11]. G.I. Ndukwe, C.M. Ojinnaka, A.O. Oyedeji, Novel bioactive triterpenoid saponin from the fruits of *Napoleonaea imperialis* P. Beauv. (Lecythidacaea), International Journal of Chemical Studies 4 (2016) 80-87.

[12]. G.I. Ndukwe, R.E. Ekong, I.R. Jack, Two new oleanane-type glycosides from *Napoleonaea imperialis* P. Beauv rind, Nigerian Journal of Chemical Research 26 (2021) 70-81.

[13]. I.R. Jack, R.E. Ekong, G.I. Ndukwe, Phytochemical constituents of dichloromethane fraction and essential oil of *Napoleonaea imperialis* rind, Journal of Pharmacognosy and Phytochemistry 9 (2020) 56-66.

[14]. G.I. Ndukwe, A. Oluah, G.K. Fekarurhobo, Isolation of an isoflavonoid and a terpenoid from the heartwood of *Baphia nitida* Lodd. (camwood), Ovidius University Annals of Chemistry 31 (2020) 5-8.

[15]. V. Kandati, P. Govardhan, C.S. Reddy, A.R. Nath, R.R. Reddy, In-vitro and in-vitro anti-inflammatory activity of *Andrographis serpyllifolia* (Rottl.Ex Vahl.), International Current Pharmaceutical Journal 1 (2012) 199-204.

[16]. V.H. Raju, S. Ganapaty, S.S. Prasanna, G.J. Vijaya, P.S. Kishore, A.K. Asif, phytochemical and pharmacological evaluation of Tragia cannabina for anti-inflammatory activity, International Current Pharmaceutical Journal 1 (2012) 213-216.

[17]. P.S. Jain, B.S. Bari, Isolation of lupeol, sigmasterol and campesterol from petroleum ether extract of woody stem of *Wrightia tinctoria*, Asian Journal of Plant Sciences 9 (2010) 163-167.

[18]. P. Kiranmayee, K. Anitha, R. Usha, Phytochemical investigation of *Caralluma attenutata* (Wright) roots, International Journal of Pharmacognosy and Phytochemical Research 7 (2015) 1120-1024.

[19]. Y. Isah, I. Ndukwe, O.J. Amupitan, Isolation of stigmasterol from aerial plant part of *Spillanthes acmella* Murr, World Journal of Life Science and Medical Research 2 (2012) 78-86.

[20]. L.L. Pierre, N.M. Moses, Isolation and characterization of stigmasterol and B-sitosterol from *Odontonema strictum* (Acanthaceae), Journal of Innovations in Pharmaceuticals and Biological Sciences 2 (2015) 88-96.

[21]. P.V. Chaturvedula, I. Prakash, Isolation of stigmasterol and sitosterol from the dichloromethane extract of *Rubus suavissimus*, International Current Pharmaceutical Journal 1 (2012) 239-242.

[22]. A.J. Yusuf, M.I. Abdullahi, A.K. Haruna, A.Y. Idris, A.M. Musa, Isolation and characterization of stigmasterol and bis-(5,7-diacetyl-catechin-4'-α-rhamnopyranoside) from the stem bark of *Neocarya macrophylla* (Sabine) Prance (Chrysobalanaceae) Nigerian Journal of Basic and Applied Science 23 (2015) 15-22.

[23]. C.J. Cayme, Y.C. Ragasa, Structure elucidation of β-stigmasterol and β-sitosterol from *Sesbania grandiflora* (Linn) Pers. and β-carotene from *Heliotropium indicum* Linn. by NMR spectroscopy, Chemical Society of the Philippines 20 (2004) 5-14.

[24]. M. Dong, X.Z. Feng, X.B. Wang, T. Ikejima, J.L. Wu, Steroidal saponnins from *Dioscorea panthaica* and their cytotoxic activity, An International Journal of Pharmaceutical Sciences 59 (2004) 294-298.

[25]. M.R. Habib, F. Nikkon, M. Rahman, M.E. Haque, M.R. Karim, Isolation of stigmasterol and beta sitosterol from methanolic extract of root of bark of C*alotropis gigantean* (Linn), Pakistan Journal of Biological Science 10 (2007) 4174-4176.

[26]. O. Gabay, C. Sanchez, C. Salvat, F. Chevy, M. Breton, G. Nourissat, Stigmasterol: A phytosterol with potential antiosteoarthritic properties, Osteoarthritis Cartilage 18 (2010) 106-116.

[27]. A.K. Batta, G. Xuab, A. Honda, T. Miyazaki, G. Salen, Stigmasterol reduces plasma cholesterol levels and inhibits hepatic synthesis and intestinal absorption in the rat, Journal of Pharmaceutical Sciences 55 (2006) 292-299.

[28]. L. Jae-Chul, H.P. Jong, B. Milos, K. Alexander, H. Yeong-Hwan, K. Byung-Soo, Antimutagenic constituents from the thorns of *Gleditsia sinensis*, Chemical and Pharmaceutical Bulletin 53 (2005) 561-564.

[29]. S. Panda, M. Jafri, A. Kar, B.K. Meheta, Thyroid inhibitory, antiperoxidative and hypoglycemic effects of stigmasterol isolated from *Butea monosperma*, Fitoterapia 80 (2009) 123-126.

[30]. M. Afzai, G. Gupta, I. Kazmi, M. Rahman, O. Afzal, J. Alam, Anti-inflammatory and analgesic potential of a novel steroidal derivatives from *Bryophyllum pinnatum*, Fitoterapia 83 (2012) 853-858.

[31]. G. Wang, D. Jiang, J. Li, J. Han, Y. Liu, X. Li, Anthelmintic activity of steroidal saponins from *Dioscorea zingiberensis*, C. H. Wright against *Dactylogyrus intermedius* (Monogenea) in goldfish (*Carassius auratus)*, Parasitology Research 107 (2010) 1365-1371.

[32]. U. Sharma, N. Kumar, B. Singh, K.R. Munshi, S. Bhalearao, Immunomodulatory active steroidal saponins from *Asparagus racemosus*, Medicinal Chemistry Research 22 (2013) 573-579.

[33]. N. Antonio, D.L.H. Beatriz, V. Angel, Anti-inflammatory and immunomodulating properties of a sterol fraction from *Sideritis foetens* CLEM,

Biological and Pharmaceutical Bulletin 24 (2001) 470-473.
[34]. T. Kawabata, M. Cui, T. Hasegawa, F. Takano, T. Ohta, Anti-inflammatory and anti-melanogenic steroidal saponin glycosides from Fenugreek (*Trigonella foenum-graecum* L.) seeds, Planta Medica 77 (2011) 705-710.
[35]. A. Li, W. Huang, Y. Wen, G. Gong, Q. Zhao, G. Yu, Anti-thrombotic activity and chemical characterization of steroidal saponins from *Dioscorea zingiberensis* CH, Fitoterapia 81 (2010) 1147-1156.

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