Phytoecdysteroids from the stem bark of *Vitex doniana* with anti-inflammatory activities

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Abstract

Ethnopharmacological significance of *Vitex doniana* (Verbenaceae) leaves and stem barks in management of stomach, rheumatic pains and inflammatory disorders has been supported by anti-inflammatory and analgesic activities studies, with no attempt to identify the active components. Consequently, chromatographic and spectroscopic procedures identified three new ecdeysteroids 21-hydroxyshidasterone (1), 11-hydroxy-20-deoxyshidasterone (2), 2, 3-acetonide-24-hydroxyecdysone (3) from the stem bark methanol extracts along with known ecdysteroids, shidasterone (4), ajugasterone C (5), 24- hydroxyecdysone- (6 hydroxyecdysone (7). The compounds [1-7]showed significant (P ≤ 0.05) inhibi

concentration on rat paw oedema development in the later phase of carrageenan-induced inflammation on Sprague Dawley rats. These results supported the previously reported anti-inflammatory activities of the plant extractives and it ethnopharmacological applications.

Keywords: *Vitex doniana, Verbenaceae, anti-inflammatory, 21-hydroxyshidasterone, 11-hydroxy-20-deoxyshidasterone , 2,3-acetonide-24-hydroxyecdysone*

Introduction

Vitex doniana (Verbenaceae) is small to medium sized tree up to 25 m tall tropical tree with no phytochemical report in spite of the available ethnopharmacological significance among different African communities [1-2], including management of stomach, rheumatic pains and inflammatory disorders [3].Antidepressant effects, potentiation of sodium thiopental sleeping time, muscle relaxant [4], anti-inflammatory and analgesic activities [1] of the leaves extract of *V. doniana* have been investigated and anti-hypertensive effect of the stem bark has been reported on normative and hypertensive rats [5]. The potent anti-inflammatory activity of the extracts of *V. doniana* with no phytopharmaceuticals associated with the activity prompted phytochemical analysis of the *V. doniana* stem bark for anti-inflammatory compounds. In this note, isolation of three new ecdysteroids [21-hydroxyshidasterone (**1**), 11-hydroxy-20-deoxyshidasterone (**2**), 2,3-acetonide-24-hydroxyecdysone (**3**)] and four known ecdysteroids [shidasterone (**4**), ajugasterone C (**5**) 24-hydroxyecdysone (**6**)11α-Hydroxyecdysone (**7**)] with comparable anti-inflammatory activities as diclofenac is reported.

Materials and Methods

Plant materials

The stem barks of *Vitex doniana* Sweet were collected from the Mau Forest, Kenya (0°29'07.70"S; 34°44' 02.28"E; elevation 4815 m a.s.l). A voucher specimen (COO-VD-2010-02) was deposited at the University of Nairobi Herbarium, Department of Botany. Identification of the plant material was done by Mr. Patrick C. Mutiso of the Department of Botany, School of Biological Science, University of Nairobi.

General instrumentation and chemicals

Optical rotations were measured with a Perkin-Elmer 341 polarimeter. UV spectra recorded with a Shimadzu UV 2101 PC spectrophotometer. NMR spectra were recorded on Bruker Avance DRX-500 apparatus (500 MHz, 125 MHz) using either CH_3OH-d_4 or DMSO-d_6 and TMS as reference for both carbon and proton. Mass spectra were performed with Bruker Apex II mass spectrometer while HRESIMS were recorded on PEG-STAR ESI-MS/MS spectrometer. IR was performed using Perkin Elmer FTIR 600 series. Silica gel 60 F_{254} TLC plates (E. Merck, Darmstadt, Germany) were used. Silica gel (Merck 60-120 Mesh ASTM) and MPLC [column: Spherisorb ODS 2-5 pm, 250 x 4.6 mm; detector: 254 nm] were used in chromatographic separation. A plethysmometer (Ugo-Basile, Varese, Italy) was used for measuring the paw volume in experimental rats. Diclofenac, carrageenan and standard rodent pellet used in anti-inflammatory experiment were obtained from Total Heathcare, Parwanoo, India, Sigma Chemicals Company, USA and Livestock Feed PLC, Lagos, Nigeria, respectively.

Extraction and isolation

Pulverized, dry stem bark (3 kg) was extracted successively with *n*-hexane and MeOH (5 L) in a soxhlet extraction apparatus. The concentrated MeOH extract (120 g) was diluted with H_2O and the

filtered dark brownish solution extracted successively with $CHCl_3$, EtOAc and BuOH (1 L each), using a continuous liquid-liquid extraction apparatus. The $CHCl_3$ extract (30 g) subjected to column chromatography (CC) on silica gel (900 g) using $CHCl_3$-MeOH as eluent, with increasing MeOH content (40-63 µm, 5 x 100 c mL fraction volumes) and two main fractions were selected by TLC examination of the eluates. The second fraction was repeatedly chromatographed to afford 70 mg of shidasterone (**4**).

The EtOAc extract (47 g) was subjected to CC on silica gel (1.2 kg), using a similar eluting solvent as that employed for the $CHCl_3$ extract and three fractions were subjected to further separation. Fraction 1 (21 g) eluted with $CHCl_3$-MeOH (19:1 to 9:1) rechromatographed on CC (40-63 µm, 5 x 50 cm, 50 m to afford a fraction (160 mg) containing an ecdysteroid according to TLC colour reactions with *p*-anisaldehyde-sulphuric acid. The impure ecdysteroids fraction (150 mg) was further purified by MPLC [column: Spherisorb ODS 2-5 pm, 250 x 4.6 mm; mobile phase: MeOH-H_2O (1:1); flow rate: 1.0 ml/min; detector: 254 nm] to furnish 32 mg of 11-hydroxy-20-deoxyshidasterone (**2**) and 41 mg of 2,3-acetonide-24-hydroxyecdysone (**3**). Fraction 2 (432 mg) eluted with $CHCl_3$-MeOH (17:3 to 4:1) crystallized in MeOH-EtOAc to afford 102 mg of 24-hydroxyecdysone (**6**) as a colourless needle-like crystals, which was identified by TLC and spectroscopic (IR and ^1H NMR) comparisons with those of authentic samples [6]. The mother liquor afforded 61 mg 21-hydroxyshidasterone (**1**) after MPLC (same conditions described earlier) purification and crystallization in MeOH-EtOAc. A white amorphous solid precipitated from fraction 3 eluted with $CHCl_3$-MeOH (4:1 to 7:3), which was then triturated using hot MeOH several times to afford 43 mg as a mixture of **6** and ajugasterone (**5**). The BuOH extract (34 g) was chromatographed on silica gel (900 g) using $CHCl_3$-MeOH as eluent, adjusting the polarity using MeOH and three major fractions were obtained. The first fractions contained the previously

isolated compounds from the EtOAc extracts **1**, **5** and **6** while the second fractions (361 mg) giving a positive colouration of ecdysteroids with *p*-anilashyde reagent on TLC was subjected to reverse-phase MPLC separation resulting to isolation of **5** (53 mg)and-hydroxyecdysone11α (**7**) (40 mg).

Compound **1** (21-hydroxyshidasterone-22,25- {(2β, epoxy-2,3,14,20, 21-pentahydroxycholest-7-en-6-one}): white crystalline solid (MeOH: EtOAc); m.p. 232-234°C (uncorrected); R_f 0.42 silica gel 60 F_{254} ($CHCl_3$/MeOH, 4:1); $[\alpha]_D^{25}$ = +13 (c = 0.1 MeOH); UV $_{max}$(MeOH)(log ε)λ 249 (2_{max}3448,.34); IR 2968, 1652, 1514, 1057 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD, 500, 125 MHz) see Table 1; ESI-MS (rel. int): 479 (50 $[M+H]^+$) 461(40 $[M+H-H_2O]^+$,), 443 (40, $[M+H-2H_2O]^+$), 425 (35, $[M+H-3H_2O]$+), 407 (100, $[M+H-4H_2O]^+$), 380 (60, $[M+H-C_6H_{11}O,C_{20}/C_{22}]^+$), 362 (30, $[M+H-C_6H_{11}O-H_2O]^+$), 344 (65, $[M+H-C_6H_{11}O-2H_2O]^+$), 326 (35, $[M+H-C_6H_{11}O-3H_2O]^+$), 308 (30, $[M+H-C_6H_{11}O-4H_2O]^+$); HR-ESI-MS (ToF) *m/z*: 501.2096 (calcd. 501.2189 $[M+Na]^+$).

Compound **2** (11-hydroxy-20-deoxyshidasterone {rel. (2β,3β,5β,11β,22R)-epoxy-2,3,11,14 - tetrahydroxycholest22,25-7-en-6-one}): white powder ($CHCl_3$/MeOH); m.p 258-262°C (uncorrected); R_f 0.65 silica gel 60 F_{254} ($CHCl_3$/MeOH, 4:1); $[\alpha]_D^{25}$ = +7 (c = 0.01 MeOH); UV (MeOH)$_{max}$(logλ ε) 249 (3.5 (KBr)$_{max}$3427,ν2832, 1654, 1059 cm^{-1}; ^{1}H and ^{13}C NMR (CD_3OD, 500, 125 MHz) see Table 1; ESI-MS (rel. int) 463 (60 $[M+H]^+$), 445 (100$[M+H-H_2O]^+$), 427 (30 $[M+H-2H_2O]^+$), 409 (60[M+H–$3H_2O]^+$); HR-ESI-MS (ToF) *m/z*: 463.3591 (calcd. 463.3454 $[M+H]^+$), 485.2651 (calcd. 485.2636 $[M+Na]^+$).

Compound **3** (2,3-acetonide-24-hydroxyecdysone-acetonide- {2β,3 14, 22R, 24,25-pentahydroxycholest-7-en-6-one}): white needles

crystals (MeOH); m.p 158-160°C (uncorrected); R_f 0.78 silica gel 60 F_{254} ($CHCl_3$/MeOH, 4:1); $[\alpha]_D^{25}$ = +56.4 (c = 0.9 MeOH); UV (MeOH)$_{max}$(logλ ε) 244 (3.$_{max}$95)3423, 2937,nm; IR (1653, 1462, 1376, 1050 cm^{-1}. ^1H and ^{13}C NMR (CD_3OD, 500, 125 MHz) see Table 1; ESI-MS (rel. int): 520.7 (20 $[M]^+$), 502 (5 $[M–H_2O]^+$), 484 (25, $[M–2H_2O]^+$), 466 (5 $[M–3H_2O]^+$), 448 (2 $[M–4H_2O]^+$), 360 (50 $[M+H–C_{20}\text{-}C_{29}\ C_8H_{17}O_3]^+$), 342 (80 $[M+H\text{-}(C_8H_{17}O_3 + H_2O)]^+$), 300 (100 $[M+H\text{-}(C_8H_{17}O_3 + CH_3C\text{-}+H_2O)]^+$), 282 (20 $[M+H–(C_8H_{17}O_3 + CH_3C\text{-}+2H_2O)]^+$), 161 (40 $[C_8H_{17}O_3]^+$), 125 (30 $[C_8H_{17}O_3 –2H_2O]^+$); HR-ESI-MS (ToF) *m/z*: 543.4561 (calcd. 543.4560 $[M+Na]^+$).

Anti-inflammatory activity

Sprague Dawley rats (140-170 g) of either sex used in this study were obtained from the Laborotory Animals Centre of the College of Medicine, University of Lagos, Nigeria. The animals were kept in well-ventilated and hygienic compartments maintained under standard environmental conditions and fed with standard rodent pellet and water *ad libitum*. The experimental procedures adopt this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [7].

Carrageenan-induced rat paw oedema: Sprague Dawley rats (140-170 g) of either sex were randomly divided into groups of 5 animals each, and were used after a 12 h fast but allowed free access to water except during the experiment. The compounds [**1**-**7**] from *Vitex doniana* (10 mg/kg/bw. p.o.), diclofenac 50 mg/kg, p.o. (Reference drug) and 0.05% DMSO in normal saline 10 ml/kg, p.o. (Control) were administered one hour before subcutaneous injection of 100 µl of carrageenan (1%$^w/_v$ in 0.9 % normal saline) into the callus of the right hind paw of the animal.

One hour after administration of the various agents, oedema was induced by injection of carrageenan (0.1 ml, 1%, w/v in saline) into

the sub-plantar tissue of the right hind paw [8]. The linear paw circumference was then measured using the cotton thread method of [9]. Measurements of paw circumference were done immediately before injection of the phlogistic agent and at 1 h interval for 24 h.

$$\text{Inhibition (\%)} = \frac{\text{Increase in paw oedema [Control]} - \text{Increase in paw oedema [treated]}}{\text{Increase in paw oedema [control]}} \times 100$$

Statistical analysis

Results obtained were expressed as mean ± standard error of mean (SEM). The data were analyzed using one way analysis of variance (ANOVA) followed by Bonferroni posttests.

Result and Discussion

A number of *Vitex* species have been investigated for phytoecdysteroids [10]. Phytoecdysteroids analysis of *Vitex* has shown the occurrence of the three common (C-27, C-28 and C-29) ecdysteroids skeletal types [11]. In the course of this study, isolation of three new ecdeysteroids, [21-hydroxyshidasterone (**1**), 11-hydroxy-20-deoxyshidasterone (**2**), 2,3-acetonide-24-hydroxyecdysone (**3**)] and four known ecdysteroids [shidasterone (**4**) [12], ajugasterone C (**5**) [13], 24-hydroxyecdysone (**6**) [14],- 11α hydroxyecdysone (**7**) [15] was achieved.

The structures of compounds **1**–**3** (Fig 1) were elucidated by using NMR, UV, IR and MS measurements. Compounds **1** and **2** both white crystalline solids displayed greenish grey spot upon spraying with *p*-anisaldehyde on TLC plate comparable to the spot for **6** signifying structural similarities. Compound **1** gave an ESI-MS molecular ion peak at *m/z* 479 consistent with the molecular formula $C_{27}H_{42}O_7$, confirmed by HR-ESI-MS pseudo-molecular ion at *m/z* 501.2197 $[M+Na]^+$ (calcd 501. 2189 $[M+Na]^+$ $C_{27}H_{42}O_7$). The characteristic fragment ions were formed from the intact parent

compound by the loss of water: *m/z* 461 $[M+H-H_2O]^+$, 443 $[M+H-2H_2O]^+$, and 425 $[M+H-3H_2O]^+$, which is a common feature in ecdysteroids mass spectra [16]. In the IR spectrum, it showed strong absorption of hydroxyl groups at 3448 cm^{-1} and characteristic absorption-unsaturatedof carbonylα, βmoiety at 1652 cm^{-1}.

Compound **2** showed UV absorption$_{max}$240nmwhichmaxima a was in accordance with the presence of the 7-en-6-one chromophore of ecdysteroids. A molecular-ion peak at *m/z* 463.6188 ($[M + H]^+$, calcd. 463. 2981 $[M+H]^+$) and pseudo molecular ion peaks at 485.2652 $[M+Na]^+$ (calcd. 485.2635 $[M+Na]^+$) of the compound indicated a molecular formula $C_{27}H_{42}O_6$, in accordance with the ^{1}H-and ^{13}C-NMR data (Table 1). In the IR spectrum, it showed strong absorption of hydroxyl groups at 3427 and 1059 cm^{-1} and characteristic - unsaturatedabsorptionketogroupat 1654of the α cm^{-1}. Compound **3** isolated as white needles, displayed a UV spectrum$_{max}$244nm) λand IR spectrum with prominent absorption bands at 3423 cm^{-1} and 1653 cm^{-1}, confirming the presence of a 7-en-6-keto group on the hydroxylated steroid nucleus. The ESIMS of **3** revealed a peak at *m/z* 520.7 for $[M]^+$, which is consistent with the molecular formula $C_{30}H_{48}O_7$, confirmed by HR-ESI-MS peak at *m/z* 543.4561 (calcd. 543.4566 $[M+Na]^+$). In the ESI mass spectrum, the peaks at *m/z* 502, 484, 466, 448 corresponded to successive loss of four molecules of water from the parent molecular ion.

For the signal assignment, four methyl signals appearing as singlets were indentified in the ^{1}H NMR (Table 1) spectrum of compound **1** contrary to the expected five methyl groups for majority of the ecdysteroids. Such an observation implied an oxygenation of one the side chain methyl groups (C-26/C-27 or C-21). The characteristic HMBC correlations (Fig. 1) of the methyl groups through two and three bonds were utilized in the assignments of the germinal Me-26 (δ_H1.28, 2H, s) and Me-27 $_H$ 1(δ.28, 2H, s) groups owing to their mutual HMBC correlation, indicating lack of oxygenation on C-26/C-27. The differentiation between Me-19 $_H$ 1(δ.05, 3H, s) and

Me-18 $_H$ 0(δ.98, 3H, s) atoms of the angular methyl groups was achieved by considering the ^3J correlation of the latter with C-17. Appearance of a $_H$ 3.54doublet(1H,*J*=12Hz) couplingsignal at to another doublet overlapping with solvent signal$_H$3.41(1H, *J* at δ = 12 Hz) in the ^{1}H NMR spectrum and HMBC cross-peaks detected between the same signal and ^{13}C NMR signal$_C$50.8)ascribed(δfor C-17 suggested an oxymethylene protons on C-21. The ^{13}C NMR chemical shift values (Table 1) of C-22 (84.0) and C-25 (82.5) and the H-22/Me-26 NOE correlation (Fig. 1) proved the presence of OR (R≠H) and a-ringfiveunitinthe memberedsidechain[17]. Further support of this structure was achieved from the comparison of its spectral data with those of shidasterone [6] except for the absence of one methyl (Me-21) instead the oxymethylene signals observed in **1.** Analysis of ^{1}H NMR spectral features and relative positions of H-2, H-3, H-5, H-7, H-9 and H-17, as well as those of Me-18 and Me-19 of **1** and **2** were almost identical. However, a notable difference was observed with presence of a methyl doublet [δ$_H$0.92 d (3H, *J* = 6.5 Hz, H-21)] and an additional oxymethine proton$_H$3.76 m[δ(1H, H-11)] in the ^{1}H NMR spectrum. Assuming the structure of compound **2**, the methyl doublet could possibly exist at C-21, implying 20-deoxyshidasterone. C-11 has been observed to be a biosynthetically labile hydroxylation point in ecdysteroids [17-18-19], with cyclization of the side chain to the tetrahydrofuran, attachment of a hydroxyl group at C-11 was the other possible difference between the two compounds. Moreover, the down-field shift observed for many signals in the ^{1}H NMR spectrum were attributed to the introduction of an 11-hydroxyl group to the ecdysteroid molecule [10]. Significant and diagnostic shifts were

observed by the presence $_H$of4.32 a carb and down-field shifts of H-9 and H-12$_{ax}$ of ca. 0.10 and 0.22,
respectively (Table 1). Further down-field shifts of the remote protons by ca. +0.32 and +O.4 were also observed for H-1$_{eq}$ and H-12$_{eq}$, respectively as compared those of compound **1**. It was noted that the presence of an 11-hydroxyl group caused a +2.1 down-field

shift of C-1 resonance, in addition to the expected down-field shift of the C-11 resonance in the ^{13}C NMR spectrum. Significant down-field shifts were also observed for C-9 and C-12 signal (ca.+3.3 and 10.6, respectively) in the ^{13}C NMR spectrum as compared to those of compound **1**.

Further support of these structures were achieved from the comparison of their spectral data with those of **4**[6], except for the absence of one methyl (Me-21) instead the oxymethylene signals observed in **1** and 11-hydroxyl; 20-deoxy for **2**. The chemical shift δ_H 85.3 for C-14 established an OH substitution on **1** and **2**, which are in accordance with a 7-en-6-one moiety, showed HMBC cross-peak with the olefinic H-7 which in turn correlated with two CH units C-5 and C-9. The HMBC ^2J coupling of the latter methine H-atoms (H-9) with the C-atom of the oxo group and the quaternary C-atom in the sp^2 hybrid state (122.6) justified their assignments of the two compounds. The H_α-9/H_α-2 and Me-19/H_β-5 correlations in the NEO spectra of **1** and **2** established a *cis* type junction of rings A and B (Fig. 1). Moreover, the presence of H_β-12/Me-18, H_β-12/CH_2OH-21 (**1**) or Me-21 (**2**) and H_α-12/H_α-17 cross-peaks and the absence of H_α-9/H $_\alpha$-15 correlation verified the *trans*-type junction of rings C and D. The NOESY plots were sufficient to identify the configuration at C-20, but insufficient to identify the configuration at C-22. Fortunately, the absolute configuration (22R) of **4** has been established [20]. Based on the biogenetic considerations, the intermolecular closure of the furanyl ring from the known precursor 20-hydroxecdysone followed by oxidation of Me-21 further implied the stereochemical arrangement of **1** and **2** must be similar to shidasterone. The structure of **1** and **2** were thus established as rel. 22,25-epoxy-2,3,14,20,21-pentahydroxycholest-7-en-6-one (named 21 hydroxyshidasterone) and rel. 22,25-epoxy-2,3,11,14-tetrahydroycholest-7-en-6-one (named 11-hydroxy-20-deoxyshidasterone), respectively.

The ^{13}C NMR spectrum of **3** (Table 2) displayed six signals for oxygenated carbons, besides that of the unsaturated ketone. Analysis of mass fragmentation and ^{13}C NMR data of **3** suggested the location of three hydroxyl groups at the side chain of this ecdysteroid. The peak at *m/z* 360 in the mass spectrum of **3** corresponded to the loss of side chain moiety to give a fragment ion bearing the ecdysteroid rings and a methyl ketal unit. The fragments ions at *m/z* 342 and 300 were due to ions produced by fission between C-17 and C-20 with loss of one molecule of water and an isopropyl unit, respectively. The assignment of the side chain hydroxyl groups at C-22, C-24 and C-25 was evident from the side chain ions *m/z* 161 and 125 derived from cleavage between C-17 and C-20 corresponding to $C_8H_{17}O_3$ and $C_8H_{17}O_3–2H_2O$, respectively. The ^{13}C NMR spectrum (Table 2) showed that the compound was quite similar to 24-hydroxyecdysone (**4)** except for three more peaks corresponding to $_C$ 108.6, 28.6, and 27.1 and signals for C-2 and C-3 shifted down field to $_C$ 75δ.1 and 72.6, respectively. The molecular mass observed at *m/z* 520 was 42 a.m.u higher than the molecular weight of **4**, indicating the additional isopropyl unit to adjacent diol functionality. For the signal assignment, six of seven methyl signals appearing as singlets were identified in the ^1H NMR spectrum (Table 1). The characteristic $^2J_{H\text{-}C}$ and $^3J_{H\text{-}C}$ HMBC correlations of the methyl groups (isopropyl moiety) were utilized in the assignment. The identification of the germinal methyl Me-26 and Me-27 groups were straightforward owing to their mutual HMBC correlation. $_H$ 1A.41 (3H,doubletd,*J*=6Hz)) indicatedpeakthe (δ presence of a secondary methyl group corresponding to 20-deoxyecdysteroid. A fact corroborated by the MS side chain fragment ions (*m/z* 161, $[C_8H_{17}O_3]$) and HMBC correlation between Me-21 to C-20 $_C$ 40(δ.6), C-17 $_C$ 51(δ.5) and in turn to C-22 $_C$ (δ 77.82). Differentiation of Me-18 and Me-19 atoms of the angular methyl groups was achieved considering the coupling of the former with C-17 and in turn with C-14 $_C$ 85(δ.7). As predicted by the MS

fragment ions, the additional $_{H}$1.19and1.45 two both s) were assigned to the isopropyl protons confirmed by their mutual HMBC correlation and in turn their $^{2}J_{H\text{-}C}$ correlation to a quaternary carbon$_{C}$108.6at.Aseriesδ of proton signals at 1.41-2.5 were attributed to resonances of overlapping methylenes and methines of the steroid framework. All of the protonated carbons were assigned by HSQC experiment. From the foregoing discussion, the structure of **3** was therefore deduced to be 2,3-acetonide-24-hydroxyecdysone.

Following the previously established anti-inflammatory activities of the crude extracts from *V. doniana* [1] that prompted the isolation of the compounds from the stem bark, the anti-inflammatory activities of these compounds were investigated and the result presented in Table 2. Injection of carrageenan into the sub-plantar tissue of the right hind paws of rats in the control group caused oedema development which peaked (0.85 ± 0.06 ml increase in paw volume) at 4 h post-phlogistic agent injection. The effect of compound **5** and **7**was significant from the 2nd to 6th with peak effect (62.68 and 60.00% inhibition, respectively) produced at the dose of 10 mg/kg at the 6th and 4th hour, respectively. The effect of compounds **3** and **6**were significant from the 4th to the 6th h with peak effect (47.76 and 71.64% inhibition, respectively) produced at the dose of 10 mg/kg at the 6th hour. The effect of compounds **1, 2** and **4**were significant from the 3rd to 6th with peak effect (68.66, 61.19 and 65.67% inhibition, respectively) produced at the dose of 10 mg/kg at the 6th hour. The peak effect for all the compounds were lesser than except for **6** (71.64%) but not significantly different (P 0≤.05) from that produced by 50 mg/kg diclofenac (70.14 % inhibition). The effects of these compounds [**1**-**7**] at 10 mg/kg and diclofenac were all time-dependent all through to the 24th h (Table 2). Carrageenan-induced inflammation consists of three distinct phases including an initial release of histamine and serotonin; a second phase mediated by kinins; and a third phase involving prostaglandins [21-20]. In this study, compound [**1**-**7**]showed significant inhibitory effect on rat

paw oedema development in the middle phase and more pronouncedly in the later phase of carrageenan-induced inflammation. This suggests that the ecdysteroids possibly acts by inhibiting the release and/or actions of vasoactive substances (histamine, serotonin and kinins) and prostaglandins. A strong involvement of effect on prostaglandins release and action is suggested based on the facts that the late phase of carrageenan-induced oedema is associated with the release of prostaglandin-like substances ([23-24] and is sensitive to clinically useful steroidal and non-steroidal anti-inflammatory agents [21].

Reference

Iwueke AV, Nwodo OFC, Okoli CO. Evaluation of the anti-inflammatory and analgesic activities of *Vitex doniana* leaves. Afri J. Biotech 2006; 5(20): 1929-1935.

Von Maydell H. *Trees and Shrubs of the Sahel: their characteristics and uses*. Verlag Josef Margaret Scientific Books, Muhestrabe 9, D-6992- Weikershein, 1990; p 397.

Irvine FR. *Woody plants of Ghana*. Oxford University Press. London; 1961; P 761-762.

Abdulrahman FI, Onyeyili PA, Sandabe UK, Ogugbuaja VO. Evaluation of the effects of the aqueous extract of *Vitex doniana* root-bark on the peripheral and central nervous system of laboratory animals. J. Appl. Sci 2007; 7(10): 1397-1403.

Olusola L, Zebulon SC, Okoye FU. Effects of *Vitex doniana* stem bark on blood pressure. Nig. J. Nat. Prod. Med 1997; 1: 19-20.

Lafont R, Harmatha J, Marion-Poll F, Dinan L, Wilson ID. Ecdybase, a free ecdysteroid database. http://www.ecdybase.org 2002.

NIH. United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research 1985.

Winter C, Risley E, Nuss O. Carrageenin-induced inflammation in the hind limb of the rat. Federation Proceedings 1962; 46: 118–126

Bamgbose SOA, Noamesi BK. Studies on cryptolepine II: inhibition of carrageenan-induced oedema by cryptolepine. Planta Medica 1981; 42: 392–396.

Suksamrarn A, Promrangsan N, Jintasirikul A. Highly oxygenated ecdysteroids from *Vitexcanescens* root bark. Phytochemistry 2000; 53: 921-924.

Filho JGS, Duringer J, Maria GLA, Tavares JF, Xavier HS, Silva MS, Cunha EVL, Filho JMB. *Vitex* species: Distribution and compilation of their ^{13}C-NMR spectral data. Chem & Biodev 2008; 5: 707-713.

Suksamrarn A, Promrangsan N, Chitkul B, Homvisasevongs S, Sirikat A. Ecdysteroids of the root bark of *Vitex canescens* PII. Phytochemistry 1997; 31: 102-107.

Zhang M, Stout MJ, Kubo I. Isolation of ecdysteroid from *Vitex strickeri* using RLCC and recycling HPLC. Phytochemistry 1992; 31: 247-250.

Coll J, Rexach N, Sanchez-Baeza F, Casas J, Camps J. New ecdysteroids from *Polypodium vulgare*. Tetrahedron 1994; 50: 7247-7252.

Suksamrarn A, Kumpun S, Yingyongnarongkul B. Ecdysteroid of *Vitex scabra* stem bark. J. Nat. Prod 2002; 65: 1690-1694.

Suksamrarn A, Sommechai C, Charulpong P, Chitkul B. Ecdysteroid from *Vitex canescens*. Phytochemistry 1995; 31: 712-718.

Simon A, Liktor-Busa E, Toth G, Kele Z, Groska J, Bathori M. Additional minor phytoecdysteroids of *Serratula wolffii*. Helve. Chim. Acta 2008; 91: 1640-1645.

Dinan L. Phytoecdysteroids: biological aspects. Phytochemistry 2001; 57: 325-339.

Ba'thori M, Pongraczi Z. Phytoecdysteroids: From isolation to their effects on humans. Curr. Med. Chem 2005; 12: 153-172.

Roussel PG, Turner NJ, Dinan L. Synthesis of shidasterone and the unambiguous determination of its configuration at C-22. J. Chem. Soc. Comm 1995; 9: 933-934.

Di Rosa M, Giroud JP, Willoughby DA. Studies of the mediators of the acute inflammatory response induced in

rats in different sites by carrageenan and turpentine. J. Pathol 1971; 104: 15-29.

Ishola IO, Akindele AJ, Adeyemi OO.Analgesic and anti-inflammatory activities of *Cnestis ferruginea* Vahl ex DC (Connaraceae) root methanolic extract. J. Ethnopharmacol 2011; 135: 55–62.

Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin oedema in rats. J. Pharmacol and Exper Therap 1969; 166: 96-103.

Zakaria ZA, Abdul Ghani ZDF, Raden-MohdNor RNS, Gopalan HK, Sulaiman MR, Mat JAM, Somchit MN, Kader AA, Ripin J. Antinociceptive, antiinflammatory, and antipyretic properties of an aqueous extract of *Dicranopteris linearis* leaves in experimental animal models. J. Nat. Med2008; 62 (2): 179-187.