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CHEMICAL CONSTITUENTS OF *OCIMUM KILIMANDSCHARICUM* GUERKE ACCLIMATIZED IN KAKAMEGA FOREST, KENYA

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ABSTRACT. The genus *Ocimum* belongs to the Lamiaceae family and is made of almost 200 species of herbs and shrubs which have potential medicinal properties. The species are native to the tropical and temperate climate zones around the globe. Two new compounds with damarane skeleton namely 2α-hydroxy-3-oxodammara-20,24-diene (**1**) and 2α,3β-dihydroxy dammara-20, 24-diene (**2**) together with apeginin7-*O*-neohespeiridoside (**3**), quercetin (**4**), turkesterone (**5**), fesitin (**6**), apeginin (**7**), chrysin (**8**), lupeol (**9**), stigmasterol (**10**), friedelin (**11**), α-amyrin acetate (**12**) and n-octacosonoic acid (**13**) are reported here from the leaves of *Ocimum kilimandscharicum*. Their structures were established on the basis of physical and spectroscopic analyses and by comparison with the literature data. Crude extracts and isolated compounds were investigated for contact toxicity and anti-feedant activity against *Sitophilus zeamais* and *Prostephanus truncatus*.

KEY WORDS: *Ocimum kilimandscharicum*, Lamiaceae, Contact toxicity, Anti-feedant activity, Acclimatized

INTRODUCTION

The genus *Ocimum,* a member of Lamiaceae family, is made up of almost 200 species of herbs and shrubs [1] which have good medicinal potentialities and are native to the tropical and temperate climate zones around the globe [2]. Many drugs commonly used today are of herbal origin [3]. *Ocimum kilimandscharicum* Guerke*,* one of the species in the genus *Ocimum*, is an economically important medicinal perennial herb widely distributed in East Africa [4] and native to Kenya [5]. It is an aromatic under shrub plant which is easily recognized by its shrubby habit, growing up to a height of eight feet [6]. Previous phytochemical studies on the plant had yielded a number of interesting compounds including steroids terpenoids [7-9] and phenolics [10], which have attracted interest on account of their anti-biotic, insecticidal, anti-oxidant and anti-carcinogenic activities [11-13]. In this communication we report the phytochemical evaluation of the leaves extract of this plant affording two new triterpenes with dammarane skeleton namely 2α-hydroxy-3-oxodammara-20,24-diene (**1**) and 2α,3β-dihydroxydammara-20, 24-diene (**2**) (Figure 1) together with eleven known ones including apeginin7-*O*-neohespeiridoside (**3**) [14], quercetin (**4**) [15], turkesterone (**5**) [16], fesitin (**6**) [17], apeginin (**7**) [18], chrysin (**8**) [19], lupeol (**9**) [20], stigmasterol (**10**) [21], friedelin (**11**) [22], α-amyrin acetate (**12**) [23] and n-octacosonoic acid (**13**) [24]. Their structures were established using physical and spectroscopic techniques as well as comparison with literature data. Compounds **1** and **2** are new while compounds **3, 6-8** and **13** are reported here from this plant for the first time.

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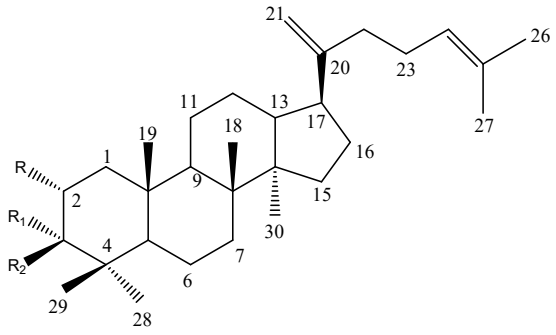

1 R = OH, $R_1 = R_2 = O$

2 $R = R_2 = OH$, $R_1 = H$

Figure 1. Structures of compounds **1** and **2**.

EXPERIMENTAL

General experimental procedure

Melting points were determined using Gallenkamp melting point apparatus (Manchester, UK). Optical rotation was measured on a Jasco P-1020 Polarimeter (Jasco Corporation, Tokyo, Japan). UV spectra were analysed using a Shimadzu UV-2401A spectrophotometer (Shimadzu Corporation, Kyoto, Japan). IR data were recorded on a Bruker Tensor 27 FTIR spectrophotometer (Bruker Corporation, Bremen, Germany) as KBr pellets. NMR data were measured in $CDCl_3$ and DMSO-d_6 on a JOEL NMR instrument operating 600 and 150 MHz, respectively. Tetramethylsilane (TMS) was used as zero reference. The mass spectral data were obtained using a Varian MAT 8200A instrument. Electron-impact (EI) mass spectra (70 eV) were measured on a Hewlett-Packard 5989B mass Spectrometer. A gas chromatography-mass spectrometry (GC-MS) Thermo Finnigan system fitted with a capillary column SPB-5MS (30 m ×0.32 mm i.d., film thickness 0.25 mm) and splitless injection was used; the oven temperature was programmed from 40 to 250 ^{o}C at 10 ^{o}C/min; injector temperature and ion source temperature were at 250 and 200 ^{o}C, respectively; MS full scan was from 50-650 ms; Helium was used as carrier gas. Silica gel 60 (63-200 μm, Merck, Darmstadt, Germany) was used for gravity column chromatography (CC). TLC was performed on precoated DC Alufoil 60 F254 sheets (Merck, Darmstadt, Germany) and detected by spraying with anisaldehyde spray reagent, UV light and iodine vapour. Paper chromatography was done on standard Whatman No 1 chromatography paper. All solvents used were of analytical grade.

Plant material collection and identification

The leaves of *Ocimum kilimandscharicum* Guerke were collected from Kakamega forest (Latitude: 0.287^{o}N and Longitude: 34, 7519^{o}E) in February 2014. The twigs were authenticated at the herbarium section, Botany Department, Maseno University where the voucher specimens are deposited under reference No. OC/2014/15732.

Plant material preparation and solvent extraction

The leaves were spread under shade for one week to dry thereafter pulverized using model 4E grinding mill. The powdered leaves (4.0 kg) were extracted with MeOH at room temperature (6 x 6 L for 7 days). The extracts were combined, filtered and evaporated under reduced pressure to give a dark green MeOH extract. The extract was separately partitioned between H_2O and *n*-hexane and EtOAc to give the soluble fractions, *n*-hexane (22.0 g green material) and EtOAc (41.5 g green material). The aqueous fraction was freeze dried to give 300.0 g brownish-green extract.

Isolation of phyto-constituents from the extracts

One part of the *n*-hexane-soluble fraction (15.0 g) was mixed with silica gel (20.0 g) in 30.0 mL dichloromethane and solvent removed in *vacuo* to obtain a green solid. This was fractionated over silica gel column (4.0 x 60.0 cm, SiO_2 300.0 g, pressure ≈ 1 bar) using *n*-hexane and *n*-hexane-EtOAc (95:5, 9:1, 6:1, 4:1 and 3:1) to give 190 fractions, each 20.0 mL. Their homogeneity was monitored by TLC (solvent systems: *n*-hexane EtOAc, 9:1 and 4:1). The sub-fraction OK 1 (*n*-hexane elution) was made up of fractions 1-20 which showed no spot on TLC. Fractions 21-75 constituted OK 2 sub-fraction (*n*-hexane-EtOAc, 95:5 elution) which produced yellow oil which lost color with time and was discarded. The OK 3 sub-fraction (*n*-hexane-EtOAc, 9:1 elution, fractions 77-100) gave a single spot R_f = 0.75 and was further purified by crystallization in CH_2Cl_2-MeOH mixture to give methyl *n*-octacosanoic acid (**13**, 120.0 mg). Fractions 102-130 (sub-fraction OK 4 from n-hexane-EtOAc elution, 1.0g) gave two spots on TLC with R_f values 0.75 and 0.65 and were separated by medium pressure chromatography to give further methyl *n*-octacosanoic acid (**13**, 20.0 mg) and α-amyrin acetate (**12**, 200.0 mg). The sub-fraction OK 5 (*n*-hexane-EtOAc, 6:1 and 4:1 elution, fractions 132-190, 2.5 g) afforded two spots of R_f values at 0.57 and 0.53 and were resolved into individual components using medium pressure chromatography (3.0 x 60.0 cm, SiO_2 90.0 g; solvent system: *n*-hexane-EtOAC, 4:1) to give friedelin (**11**, R_f=0.57, 90.0 mg) and stigmasterol (**10**, R_f= 0.53, 100.0 mg).

The ethyl acetate extract approximately 35.0 g was subjected to pass over silica gel column (5.0 x 60.0 cm, silica gel 500.0 g, pressure ≈ 1.0 bar) using *n*-hexane-ethyl acetate (10% increment of ethyl acetate), ethyl acetate neat and finally with CH_2Cl_2-MeOH (with 0.5 and 1% increment of MeOH). Three hundred and sixty fractions (each 20.0 mL) were sampled and their composition similarity monitored by TLC; eluent: *n*-hexane-ethyl acetate (4:1, 3:2 and 1:1) and CH_2Cl_2-MeOH (99:1, 98:2 and 97:3). Those exhibiting similar TLC profiles were combined resulting into five major pools (OK 6-OK 10). Pool OK 6 (fractions 20–80, 3.5 g), upon removal of solvent, afforded a yellow oily paste which lost color with time and was discarded. Pool OK 7 (fractions 83–120, 6.0 g) was similarly subjected to silica gel column and elution with *n*-hexane-ethyl acetate (4:1) followed by (3:2) affording 70 fractions of 20.0 mL each, which were combined into two major pools (OK i and OK ii) depending on the TLC profiles. Pool OK i contained two spots which upon further purification gave stigmasterol (**10,** R_f = 0.53**,** 55.0 mg) and lupeol (**9**, R_f = 0.48, 89.0 mg). Fraction OK ii (1.5 g) on evaporation of the solvent crystallized out to give white powder, which upon re-crystallization (n-hexane-EtOAc, 3:2) gave 2α-hydroxy-3-oxodammara-20,24 diene (**1**, R_f = 0.43, 75.0 mg). Fractions 125–250 constituted pool OK 8 (7.0 g), which showed two spots of R_f values at 0.48 and 0.35 (eluent: *n*-hexane-EtOAc, 3:2). Repeated medium pressure chromatography using *n*-hexane-EtOAc (4:1 followed by 3:2) afforded lupeol (**9**, R_f = 0.48, 70.0 mg) and 2α,3β-dihydroxydammara-20,24-diene (**2**, R_f = 0.35, 43.0 mg). Fractions 253-347 (pool OK 9 (8.5 g) showed three spots with R_f values at 0.51, 0.44 and 0.37 on TLC using CH_2Cl_2-MeOH (99:1). The constituents of this pool were separated using medium pressure chromatography [eluent: *n*-hexane-EtOAc (1:1) followed by CH_2Cl_2-MeOH (99:1)] to give chrysin (**8**, R_f = 0.51, 35.0 mg), apeginin (**7**, R_f = 0.44, 41.0 mg) and fesitin (**6**, R_f = 0.37, 24.0 mg).

Approximately 100.0 g of the freeze dried MeOH/water soluble fraction of the extract was chromatographed over silica gel column (5.0 x 80.0 cm, SiO_2 500.0 g, pressure ≈ 1.5 bar) using CH_2Cl_2-MeOH (10% increment of MeOH) and MeOH neat to give a total of 200 fractions (each 50.0 mL). Their homogeneity was monitored by TLC using CH_2Cl_2-MeOH (98:2, 97:3, 95:5, 9:1, and 4:1) and fractions were grouped into two pools (OK 10 and OK 11) depending on TLC profile. Pool OK 10 (fractions 15–90, 2.0 g) on repeated medium pressure chromatography separation using CH_2Cl_2-MeOH (98:2) gave turkesterone (**5**, R_f = 0.31, 44.0 mg; eluent: CH_2Cl_2-MeOH, 97:3). Pool OK 11 (9.0 g) was repeatedly chromatographed over 2% oxalic acid deactivated silica gel column using CH_2Cl_2-MeOH (95:5, 9:1 and 4:1) to yield quercetin (**4**, R_f = 0.26, 79.0 mg) and apeginin7-*O*-neohesperidoside (**3**, R_f = 0.19, 41.0 mg).

Acid hydrolysis of apeginin7-O-neohesperidoside (**3**)

Compound **3** (10.0 mg) in a mixture of 2% HCl (2.0 mL) and MeOH (20.0 mL) was heated under reflux for 2 h. The reaction mixture was reduced under pressure to dryness, dissolved in H_2O (2.0 mL) and neutralized with NaOH. The neutralized product was then subjected to TLC analysis (eluent: EtOAc-MeOH-H_2O-HOAc, 6:2:1:1) and paper chromatography (PC) (eluent: *n*-BuOH-HOAc-H_2O, 4:1:5). The sugar chromatograms were sprayed with aniline hydrogen phthalate followed by heating at 100 °C and they were identified after comparison with authentic samples.

2α-Hydroxy-3-oxodammara-20,24-diene

A white powder from CH_2Cl_2-MeOH (9:1); mp 181–183 °C; $[\alpha]^{25}_D$ + 19° (c 0.1, MeOH); IR ν_{max} (KBr) cm^{-1}: 3357, 2930, 1680, 1589, 1636, 1454, 1121; ^1H and ^{13}C NMR spectral data ($CDCl_3$, 600 MHz) see Table 1; EI-MS (rel. int.) *m/z* 440 $[M]^+$ (2), 425 (3), 391 (4), 390 (12), 364 (15), 362 (36), 336 (12), 308 (2), 249 (16), 219 (100), 163 (88), 134 (40), 105 (16), 91 (9); ESI-MS *m/z* 463.4 $[M+Na]^+$. HRMS *m/z:* 440.36550 (calcd. for $C_{30}H_{48}O_2$, 440.36543).

2α,3β-Dihydroxydammara-20,24-diene

A white powder from CH_2Cl_2-MeOH mixture with mp 190-193 °C; $[\alpha]^{25}_D$ + 59° (c 0.1, MeOH); IR ν_{max} (KBr) cm^{-1}: 3340, 2934, 1636, 1589, 1454, 1121; ^1H and ^{13}C NMR spectral data ($CDCl_3$, 600 MHz) see Table 1; ESI-MS *m/z* 465.4 $[M+Na]^+$; HRESI-MS 465.3430 $[M + Na]^+$ (calcd. for $C_{30}H_{48}O_2$+Na, 465.3424).

Contact toxicity of plant leaf extracts

The experiment tested the hypothesis that topically applied plant extracts and pure isolates solutions exhibit contact toxicity to the adult maize weevil *Sitophilus zeamais* (Curculionidae) and the adult larger grain borer *Prostephanus truncatus* (Bostrichidae). The contact toxicity assay on leaf extracts and pure isolates of *O. kilimandscharicum* were carried out according to a method previously described [25] by topical application using 3rd instar larvae. The extract stock suspensions and pure isolate solutions were prepared immediately prior to the assays by dissolving them in acetone to obtain solutions of concentrations 100.0, 300.0, 500.0 and 1000.0 µg/mL. Pure isolates were dissolved in acetone to obtain solutions of concentrations 50.0, 100.0, 250.0 and 500.0 µg/mL. The experiment was done with two replicates. For each replicate, 10 larvae were transferred to a Whatman No. 1 filter paper disc in a 90.0 mm disposable Petri dish. Larvae were treated topically with a 1.0 µL droplet of the respective solution applied onto the pronotum using a Hamilton's syringe (700 series, Microliter TM Hamilton Company, USA). For the negative control, larvae were exposed to 1.0 µL of acetone, for the positive control

larvae were exposed to 0.5 µL of deltamethrine, and for the test runs the larvae were exposed to 0.5 µL of acetone solution of each concentration. After treatment the larvae were confined in Petri dishes containing 5 corn kernels each-within metal rings and maintained in the dark at 26±2 °C and 60±5% relative humidity on a 16:8 (L:D) photocycle for 48 h, after which mortality was assessed. The percentage mortality values were subjected to analysis of variance (ANOVA). The LC_{50} values, which is the concentration at which 50% of the larvae died, the confidence upper and lower limits, the regression equations and chi-square ($\chi 2$) values were calculated using probit analysis [26].

Feeding deterrence assay

The activity of extracts and pure isolates was studied using leaf disc feeding deterrence choice bioassay method [27]. The experiment tested the hypothesis that larval feeding is deterred by plant extracts and pure isolates incorporated in fresh maize leaf discs (1350.0 mm^2). Crude extracts were tested at 2000.0 µg/mL and pure isolates at 100.0, 200.0, 500.0 and 1000.0 µg/mL. Larvae were exposed to leaf discs treated with a plant extract or pure compound versus an equal amount of solvent treated (control) diet in Petri dishes of 50.0 mm diameter. Each extract and concentration treatment combination was tested individually versus the control. The larvae were inserted into Petri dishes individually on a piece of Whatman N0 1 filter paper (1.0 x 1.0 cm) and placed centrally on portions of either treated or control diet pieces. Larvae were incubated for 72 h at 25 ± 2 ºC and on a 16:8 light:dark (L:D) photocycle. In the case of the extracts and pure isolates treatment 100.0 µL of each concentration was added to the diet. For the negative control treatment, 100.0 µL of HPLC grade acetone was added to the diet while for the positive control, 100 µL of azadirachtin was added to the diet. The insects were allowed to feed on treated discs for 24 hours. At the end of the experiment, unconsumed area of leaf disc was measured with the aid of a leaf area meter and per cent antifeedant activity calculated [28] and data subjected to analysis of variance. Each experiment was repeated three times. Insect mortality was also recorded.

Percent antifeedant activity =

$$\frac{(\text{Leaf disc consumed by the insect in control} - \text{Leaf disc consumed by the insect in treated}) \times 100}{\text{Leaf disc consumed by the isect in control} + \text{Leaf disc consumed by the insect in treated}}$$

$$\text{Percent insect mortality} = \frac{\text{Number of dead insect x 100}}{\text{Total number of treated insect}}$$

Data analyses

For the initial screening bioassay, data were corrected for mortality in the controls using Abbott's formula [29] and then normalized using an arcsine transformation. Transformed data were submitted to a randomized complete block analysis of variance (ANOVA) ($p < 0.05$) and differences between treatments were compared using Tukey's test ($p < 0.05$). For the feeding deterrence choice assay, the numbers of larvae feeding on extract-treated or pure compound-treated versus control portions of the diet were compared.

RESULTS AND DISCUSSIONS

The methanol extract from the dry pulverized leaves of *O. kilimandscharicum* was partitioned between H_2O, *n*-hexane and EtOAc. The EtOAc extract was subjected to silica gel column chromatography and gave several fractions using *n*-hexane-EtOAc mixtures with increasing

polarity of the more polar solvent. The eluants from the n-hexane-EtOAc (3:2) showed three spots of R_f values 0.43, on TLC (solvent system: n-hexane-EtOAc, 3:1). This fraction on repeated column chromatography using the same solvent system followed by *n*-hexane-EtOAc, (3:2) yielded compound **1** (R_f = 0.43) as one of the components.

Compound **1**

This was isolated as an amorphous white powder with mp 181-183 ^oC. Its IR spectrum showed absorption peaks for hydroxyl (3357 cm^{-1}), keto (1680 cm^{-1}) and double bond (1598 cm^{-1}) functional groups. The high resolution electron mass spectrum exhibited a molecular ion peak at *m/z* 440.36550 $[M]^+$ which was further supported by ESI-MS peak at *m/z* 463.3 $[M+Na]^+$ suggesting that compound **1** had the formula $C_{30}H_{48}O_2$ which corresponded to seven degrees of unsaturation. The EI-MS on the other hand gave other significant peaks at *m/z* 425 $[M-Me]^+$, 391 $[M-Me-2H_2O]^+$, 362 $[391-2Me]^+$which are characteristic of tetracyclic triterpene alcohols with di-unsaturated side chain and also a saturated skeleton [30]. The ^1H NMR data of compound **1** (Table 1), which were compared with those of the known compounds dammara-20,24-dien-3β-ol [31], dammaradienone [32] and 2-oxo-3β,19α-dihydroxyolean-12-en-28-oic [33] showed the presence of a vinylic proton on a tri-substituted double bond at δ_H 5.12 (d, *J* = 6.4 Hz), two proton doublets resonating at δ_H 4.70 (*J* = 1.4 Hz) and 4.60 (*J* = 1.4 Hz) assignable to C-21 methylene protons and two vinylic methyl protons at δ_H 1.68 (Me-26 and Me-27). The vinylic proton at δ_H 5.12 was shown to allylically couple with the vinylic methyls by homonuclear decoupling experiments, thus authenticating the presence of a terminal $–CH_2$-CH= $C(CH_3)_2$ group [31]. Furthermore, the spectrum exhibited five quaternary methyls at δ_H 0.79, 0.81, 0.94, 1.14 and 1.40 which were ascribable to C-19, C-18, C-30, C-29 and C-28 methyl protons, respectively. An up field double of doublets in the aliphatic region which resonated at δ_H 2.84 was assigned to C-2 proton and its orientation was suggested to be α-on the basis of the coupling constant, *J* =12.0, 3.8 Hz [34, 35]. Thus, this inferred that the hydroxyl functionality in this position was in β-orientation. The ^{13}C NMR spectrum (Table 1) displayed a total of 30 carbon resonances, their multiplicities assigned using DEPT-135 establishing the presence of 7 methyls, 10 methylenes (including one olefinic carbon), six methines (including an oxymethine at δ_C 69.7 and one olefinic methine at δ_C 124.4) and seven quaternary carbons including a keto group at δ_C 216.7. In fact, the ^{13}C NMR of compound **1** was similar to those of dammara-20,24-dien-3β-ol [31] with notable differences being the presence of an oxo group on ring A. In compound **1**, the C-3 hydroxyl group was replaced by a keto functionality and the positions of the hydroxyl and the keto groups on ring A were ascertained from HMQC and HMBC experiments showing one-bond H, C- and three bond H, C- correlations, respectively (Figure 2). In this way it was proved that the hydroxyl moiety was at C-2 and the keto group was at C-3, respectively, a fact cemented by NOESY correlations as outlined in Figure 2. Thus, on the basis of spectroscopic data as well as comparison with literature data, compound **1** was elucidated as 2α-hydroxy-3-oxodammar-20, 24-diene.

Compound **2**

The compound was obtained as white crystals from CH_2Cl_2-MeOH mixture. Its IR spectrum revealed the presence of hydroxyl (3340 cm^{-1}) and carbon-carbon double bond (1589 cm^{-1}) functionalities. The compound exhibited an HRESI-MS molecular ion peak at *m/z* 465.3430 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_2$+Na, 465.3424). The ^{13}C NMR spectrum (Table 1) revealed the presence of 30 carbon signals including CH_3 x 7, >CH-OH x 2, >CH- x 4, >C=CH_2 x 1, >CH_2- x 9, >C< x 4, -CH=C< x 2 as evidenced by DEPT-135 spectrum. Comparison of the ^1H and ^{13}C NMR spectral data of compound **2** with those of **1** revealed close similarities with notable difference being the replacement of the oxo group by hydroxyl functionality in compound **2** as

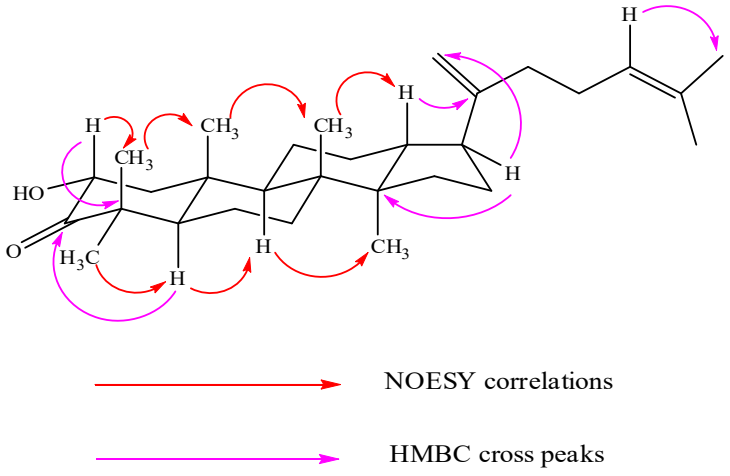

Figure 2. Selected HMBC and NOESY correlations of compound **1**.

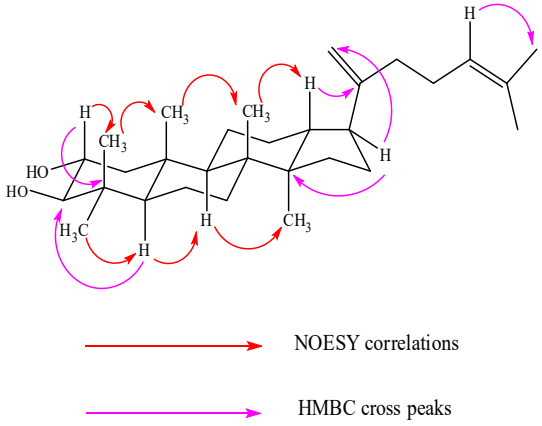

Figure 3. Selected HMBC and NOESY correlations of compound **2**.

substantiated by the molecular ion peak which is 2 atomic mass units more than that of compound **1**. Furthermore, from the ^{1}H NMR spectrum, the methine bearing hydroxyl group exhibited a doublet at δ_H3.23 (d, J = 9.0 Hz) assignable to H-3α on the basis of the coupling constant characteristic of H-3α and H-2β axial-equatorial interaction [36], a fact further evidenced by HMBC correlations between the non-protonated carbon C-10 (δ_C 39.6) and H-2 (δ_H 2.81, m). The methine proton H-2 in turn also showed cross-peak with the C-4 (δ_C 38.8). Again comparing both the ^{1}H and ^{13}C NMR data of compound **2** with those of dammara-20,24-dien-3β-ol [30] revealed a shift of a C-1 methylene peak δ_C 43.0 with corresponding ^{1}H NMR peaks at δ_H 2.20 (m) and 1.01 (m). This suggests that the second hydroxyl group was possibly at C-2, a fact augmented by COSY spectrum which revealed the ^{1}H-^{1}H proximity between H-2 and H-3. Additional evidence from comparative studies between the two structures revealed the presence of an exomethylene with ^{1}H NMR peaks appearing at δ_H 4.70 (d, J = 1.5 Hz) and 4.58 (d, J = 1.5 Hz). Its position on the side chain was ascertained by HMBC cross-peaks between H-17 (δ_H 1.75, m) and C-21 (δ_C 108.0). Thus, on the basis of spectroscopic evidence as well as

comparison with various literature data [31], compound **2** was established to be 2α,3β-dihydroxydammar-20,24-diene.

Table 1. ^{1}H and ^{13}C NMR ($CDCl_3$) of compounds **1** and **2**.

1			**2**	
C#	^{1}Hmultiplicity (*J* in Hz)	^{13}C	^{1}Hmultiplicity (*J* in Hz)	^{13}C
1	2.24 (1H, m), 1.07 (1H, m)	43.1	2.20 (1H, m), 1.01 (1H, m)	43.0
2	2.84(1H, dd, *J* = 12.0, 3.8)	69.7	2.81 (1H, d, *J* = 9.0)	69.2
3		216.7	3.30 (1H, m)	79.1
4		38.9		38.8
5	0.84(1H, dd, *J* = 11.5, 2.4)	55.9	0.90 (1H, dd, *J* = 12.0, 2.2)	56.6
6	1.62 (1H, m), 1.53 (1H, m)	21.7	1.64 (1H, m), 1.54 (1H, m)	21.3
7	1.57 (1H, m), 1.27 (1H, m)	35.5	1.60 (1H, m), 1.36 (1H, m)	35.5
8		42.2		42.1
9	1.54 (1H, m)	54.7	1.53 (1H, m)	55.2
10		39.9		39.6
11	1.90 (1H, m) 1.42 (1H, m)	22.4	1.86 (1H, m), 1.30 (1H, m)	22.3
12	1.75 (1H, m) 1.58 (1H, m)	25.2	1.79 (1H, m), 1.64-1.60 (1H, m)	25.0
13	1.84 (1H, m)	42.2	1.85 (1H, m)	41.5
14		50.7		49.0
15	1.52 (1H, m) 1.00 (1H, m)	29.8	1.53 (1H, m), 1.07 (1H, m)	29.8
16	1.87 (1H, m) 1.25 (1H, m)	27.5	1.90 (1H, m) 1.25 (1H, m)	27.2
17	1.80 (1H, m)	47.7	1.75 (1H, m)	48.0
18	0.81, s	17.0	0.90,s	16.8
19	0.79, s	16.9	0.80,s	17.0
20		150.8		150.9
21	4.70 (d, *J* = 1.4) 4.60 (d, *J* = 1.4)	109.4	4.70 (d, *J* = 1.5) 4.58 (d, *J* = 1.5)	108.0
22	1.57 (1H, m) 1.40 (1H, m)	40.0	1.60 (1H, m), 1.47 (1H, m)	40.1
23	2.17 (1H, m) 2.10 (1H, m)	27.3	2.00 (1H, m), 1.90 (1H, m)	27.5
24	5.12 (1H, t, *J* = 6.4)	124.4	5.12 (1H, t, *J* = 6.6)	124.0
25		133.6		132.0
26	1.68,s	26.6	1.68, s	26.7
27	1.68,s	18.0	1.68, s	18.4
28	1.40,s	28.7	1.43, s	28.2
29	1. 14,s	23.4	1.11, s	21.4
30	0.94,s	17.5	0.96, s	17.5

Contact toxicity assay using n-hexane, EtOAc and aqueous MeOH extracts of *O. kilimandscharicum* against insect pests *S. zeamais* and *P. trunctus* showed that the activities were dependent on concentration and time of exposure. Significant mortalities ($p < 0.05$) were observed for both the post harvest pests (Figure 3). The aqueous MeOH extract exhibited the highest activity ($p \leq 0.05$) with an Lc_{50} of 22.55±1.57 µg/mL followed by EtOAc extract with an LC_{50} of 78.05±1.26 µg/mL against *S. zeamais*. The same extracts when tested on *P. truncatus*

gave LC_{50} values of 31.95±1.35 and 56.29±1.23 µg/mL, respectively. The results showed that the extracts exhibit different levels of mortality against *S. zeamais* and *P. truncatus* at different concentrations which could be attributed to the chemical constituents in the extracts. Chemical compounds present in extracts are known to often act synergistically against physiology of many insects [37]. A previous study on the blend effect of essential oil constituents of *O. kilimandscharicum* on the two post harvest pests, *S. zeamais* and *P. truncatus* showed that the toxicity could be attributed to combined effect of the different compounds [37]. From the probit analysis, the LC_{50} values of 22.55±1.57 µg/mL and 31.95±1.35 µg/mL from aqueous MeOH extract for *S. zeamais* and *P. truncatus*, respectively compared favorably with an LC_{50} of 38.05 µL/40 g grain [37].

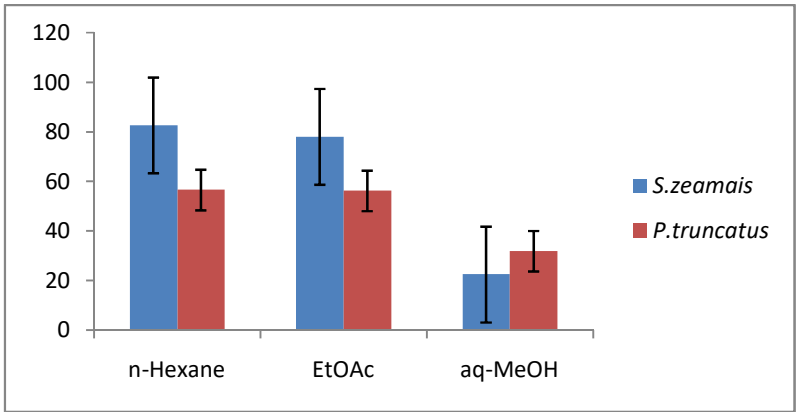

Figure 4. Contact toxicity assay results for extracts from leaves of *O. kilimandscharicum* against *S. zeamais* and *P. truncates*.

In the antifeedant activity assay of the crude extracts of *O. kilimandscharicum* against *S. zeamais* and *P. truncatus*, all the three extracts were active against the two post harvest insects with the activities increasing with increase in concentrations (Figure 4). The aqueous MeOH extract exhibited promising antifeedant activity against. *S. zeamais* and *P. truncatus* with an AFI_{50} (concentration that causes 50% feeding deterrence) values of 26.39±1.43 µg/mL and 31.85±1.23 µg/mL. The results obtained in this study compare favorably with previous investigations [38] which showed that methanol and EtOH extracts *O. kilimandscharicum* had high activity against *Sitophilus granarius* also known as the grain weevil or granary weevil. These findings also suggest that the cumulative effect of various chemical constituents present in the extracts is responsible for the activity.

When the pure isolates of *O. kilimandscharicum* were tested against *S. zeamais* and *P. truncatus* for their contact toxicities, some of them showed promising activities against the two post harvest insects, though most of the activities were not significantly different ($p > 0.05$). Turkesterone (**5**) had the highest activity against both *S. zeamais* and *P. truncatus* at LC_{50} 21.64±1.25 µg/mL and 16.06±1.34 µg/mL, respectively. Other compounds that exhibited relatively moderate activities against the pests included apeginin7-*O*-neohesperidoside (**3**) with LC_{50} value of 23.41±1.42 µg/mL and 26.20 µg/mL, respectively. The two new compounds **1** and **2** were also fairly active (Figure 5). The relatively high contact toxicity assay result of turkesterone (**5**) is in agreement with the results reported previously for this compound in reference with [39], which showed promising efficacy against *Callosobruchus maculatus*.

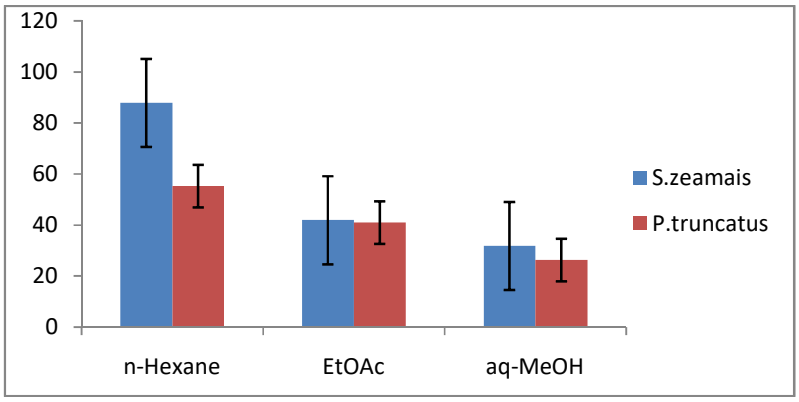

Figure 5. Antifeedant assay results for extracts from leaves of *O. kilimandscharicum* against *S. zeamais* and *P. truncates*.

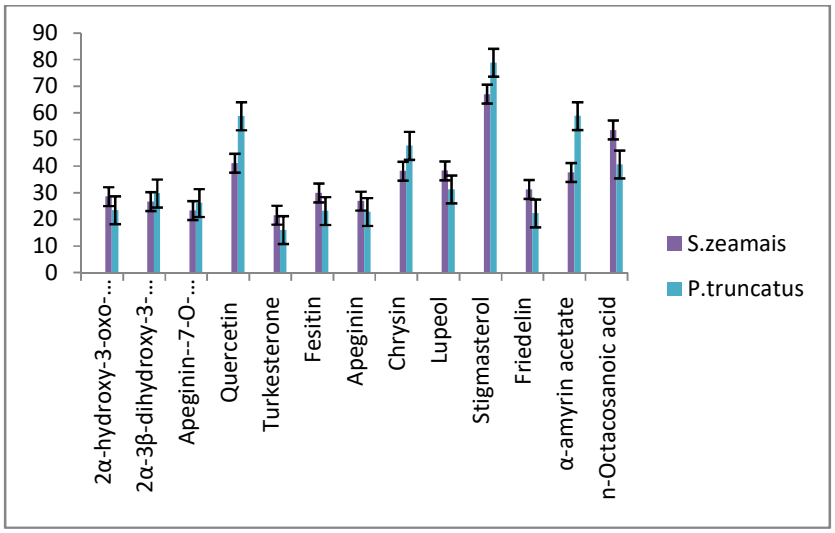

Figure 6. Contact toxicity assay results for pure compounds from leaves of *O. kilimandscharicum* against *S. zeamais* and *P. Truncates*.

Pure isolates from *O. kilimandscharicum* exhibited interesting antifeedant activities against *S. zeamais* and *P. truncatus.* The activities were concentration dependent, increasing with increase in concentration. Activities of the compounds against the two insects were not significantly different ($p > 0.05$). Turkesterone (**5**) showed the highest activities with AFI_{50} 17.50±1.73 µg/mL and AFI_{50} 21.33±1.74 µg/mL against *S. zeamais* and *P. truncatus* respectively (Figure 6) and are comparable to previous results [40].

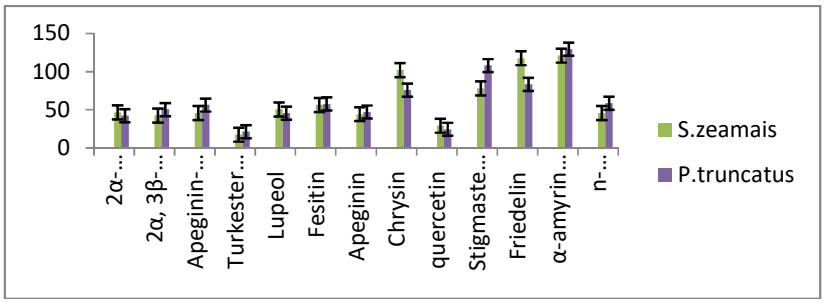

Figure 7. Antifeedant assay results for pure compounds from leaves of *O. kilimandscharicum* against *S. zeamais* and *P. truncates*.

CONCLUSION

From the leaves of the plant *Ocimum kilimandscharicum*, two new triterpenes with both contact toxicity and antifeedant activities have been isolated. This manifests the use of the plant in post - harvest grain protection.

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