



A new ent-clerodane diterpene from *Detarium microcarpum* Guill. & Perr. and its protective potential for osteoporosis

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ABSTRACT

A new clerodane diterpene, named 6 α -hydroxy-3,13E-clerodien-15-oic acid (1), together with a known clerodane diterpene (2), four known labdane diterpenes (3–6), a triterpenoid (7), a known steroid (8), and two benzenoid compounds (9 and 10) were isolated from *Detarium microcarpum* Guill. & Perr. The structures of all obtained compounds were determined by chemical properties and spectroscopic evidence, accompanied by comparisons with data in the literature. Electronic circular dichroism (ECD) was performed for compounds 1–4 to confirm the absolute configuration. Compounds 1–3 and 8–10 were evaluated for the protective effect on osteoblasts. Compound 1 was observed to increase the proliferation of dexamethasone (DEX)-treated MC3T3-E1 cells significantly at 1 μ M, which was comparable with the positive control geniposide at 10 μ M. The results were further confirmed by flow cytometry analysis. In addition, compound 1 increased the level of alkaline phosphatase (ALP) and mineralization in osteoblasts inhibited by DEX. Moreover, Compound 9 (vanillic acid) showed a pronounced inhibition (IC₅₀ 6.5 $\pm$ 0.6 μ M) on reactive oxygen species (ROS) production, and 10 (4-O-methyl gallic acid) showed a good inhibition with IC₅₀ as 103.3 $\pm$ 2.2 μ M, compared with the standard drug ibuprofen (IC₅₀ 54.2 $\pm$ 9.2 μ M). Besides, compounds 1–3 and 8–10 were non-cytotoxic against MCF-7, NCI-H460, Hela, and BJ cell lines.

1. Introduction

The genus *Detarium* (family Fabaceae) is native to Africa, comprised of only four accepted species (World Flora Online). Among which *Detarium microcarpum* Guill. & Perr. is famous for its medicinal uses by different parts of the plant to treat various diseases, such as dizziness, epilepsy, schizophrenia, paralysis, pains, typhoid fever, jaundice, dysentery, malaria, meningitis, tuberculosis, skin infection, syphilis, pneumonia, and pregnancy disorders [1–8]. Previous studies on the

constituents from the bark, leaves, and fruit of *D. microcarpum* have demonstrated the presence of different types of diterpenes [9–12], benzoylated carbohydrates [13], flavonoids [9], triterpenoids, steroids [14], isovaleronitrile and benzoic acid derivatives [4], etc. Extracts of stem bark, leaves, fruits, and seeds coat of *D. microcarpum* were verified to have antiviral [15], antibacterial [16,17], anticancer [18–20], antioxidant, genoprotective [21], sedative [8], and antidiabetic effects [22]. Some isolated compounds from *D. microcarpum* were also confirmed as active, such as diterpenes showed antifungal activity and inhibition

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properties of acetylcholinesterase [11]. An isovaleronitrile glycoside possessed antibacterial activity [4].

In the present study, a further investigation on *D. microcarpum* was conducted to discover more active natural products. Finally, a new (1) and a known clerodane diterpenes (2), four known labdane diterpenes (3–6), along with four other compounds (7–10) (Fig. 1) were isolated from the ethyl acetate extract of *D. microcarpum* stem bark. Interestingly, the new diterpene (1) showed a pronounced protective potential on osteoporosis.

Osteoporosis is a global and multifaceted bone disorder that affects the strengths and structures of bones. More than 200 million population suffer from osteoporosis. Generally, it affects women and men of all races. Osteoporosis occurs when the density and mass of bones decrease, thus increasing the risk of fractures. These fractures are associated with reduced quality of life, increased morbidity, and in the case of pelvic, hip, and spine fractures increased mortality. As compared to other health disorders, the health and social care burden due to osteoporosis is substantial [23–25].

Glucocorticoids (GCs) played key roles in the therapy of various ailments, such as asthma, inflammatory and autoimmune disorders [26]. Meanwhile, long-term consumption of GCs can commonly induce secondary osteoporosis, which is the leading cause of 30–50% of long-term GCs consumers have a high risk of fracture. However, the available clinical approach against this complication remains limited [27,28]. Natural compounds, such as geniposide, have been proved to have a potent effect on GCs-induced osteoblast apoptosis [27,29,30]. A few diterpenes have been also approved to be effective [31–33]. Interestingly, the new diterpene (1) obtained from *D. microcarpum* in this study was found to be more potent than geniposide, which has been considered as a lead candidate and used as the standard in this study.

2. Materials and methods

2.1. General experimental procedures

The Low- and High-resolution EI-MS spectra were recorded on JEOL MS route JMS 600H and Thermo Finnigan MAT 95XP, respectively. NMR spectra were recorded on Bruker Avance Neo and conducted on 500 and 600 MHz Infrared (IR) spectroscopy technique was performed by an FTIR-Bruker vector 22 (Fourier Transform Infrared Spectrophotometer) in KBr disk. UV-Visible technique and Melting point were performed on UV-Visible Spectrophotometer (Thermo scientific) and Buchi apparatus of melting respectively. Optical rotation and electronic circular dichroism (ECD) spectra were measured on Polarimeter JASCO P-200 and Circular Dichroism Spectro-Polarimeter JASCO-J-810 respectively.

2.2. Plant material

The stem bark of *Detarium microcarpum* Guill. & Perr. was obtained from the campus of the University of Ilorin, Kwara State, Nigeria, in August in 2017. The material was authenticated at the Forest Herbarium Ibadan (FHI) in Forestry Research Institute of Nigeria (FRIN), where voucher specimens were deposited with a voucher number FHI 111953.

2.3. Extraction and isolation

A total of 6.5 kg dry stem bark of *D. microcarpum* was extracted by methanol via soaking. The combined and concentrated crude extract was reextracted by hexane, ethyl acetate, *n*-butanol, successively, resulting in the ethyl-acetate fraction (140 g). It was subjected to fractionation using silica gel column chromatography (CC) with a gradient elution of ($\text{CH}_2\text{Cl}_2/\text{MeOH}$) to afford 17 fractions. Fr-1 (1.08 g), Fr-3 (0.55 g), Fr-4 (0.36 g), and Fr-5 (0.16 g) were loaded to silica gel CC and

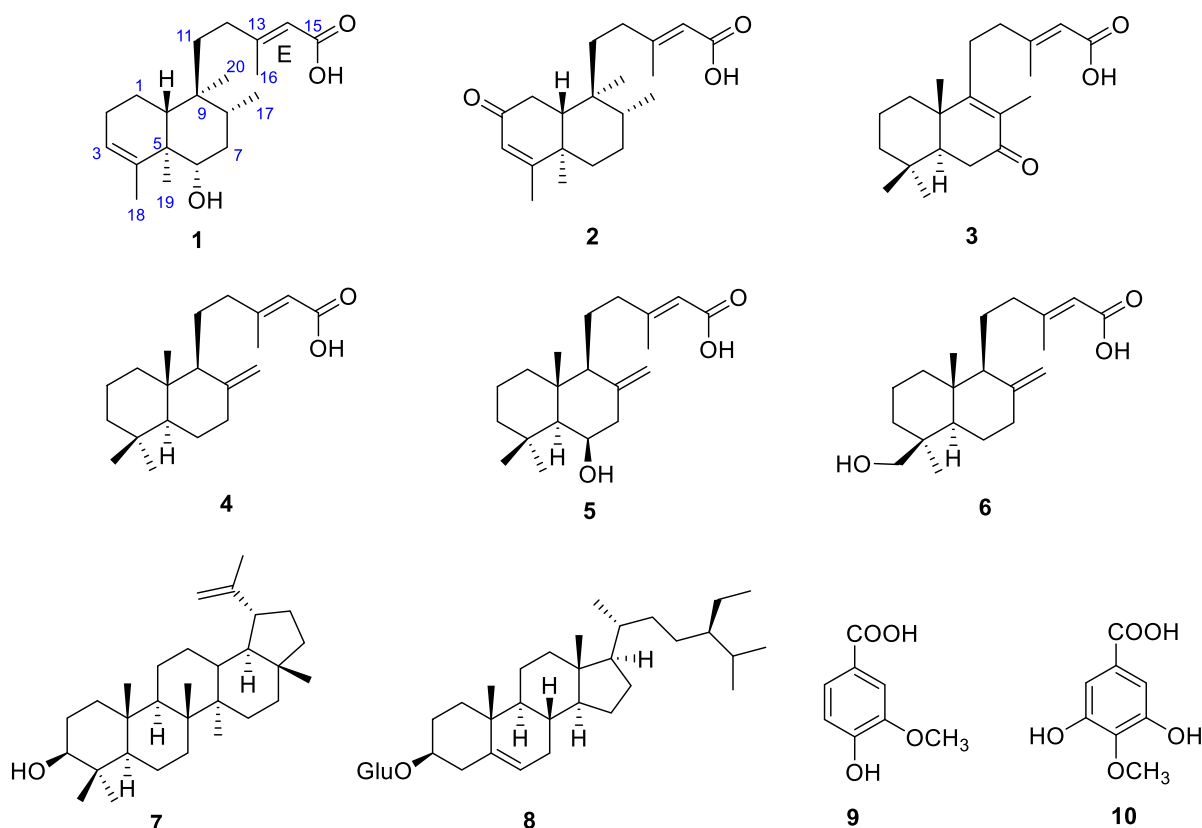


Fig. 1. Structures of compounds 1–10.

eluted with a gradient elution of Hexane/Acetone to obtain 13, 8, 8, and 11 sub-fractions, respectively. Whereas, the fractions Fr-6 (0.92 g), Fr-8 (2.62 g), and Fr-9 (0.83 g) were loaded on reversed-phase C18 silica gel CC with gradient elution of 20%, 40%, 60%, 80%, and 100% MeOH in H₂O to afford 10, 7, and 7 sub-fractions, respectively. Compound **7** (3 mg) was crystallized from Fr-1–2 (15 mg). Fr-3–1 (210 mg) and Fr-4–3 (132 mg) were applied to silica gel CC to yield 5 and 7 sub-fractions, respectively. Fr-3–1–2 (120 mg) was further separated through silica gel CC using a gradient elution of Hexane/EtOAc to obtain six sub-fractions. Fr-3–1–2–6 (26 mg) was purified via NP-HPLC using Hexane/EtOAc (90/10) as a mobile phase to get compound **4** (3.7 mg). Fr-4–3–4 (17.1 mg) was subjected to NP-HPLC by the system of Hexane/EtOAc (75:25) to yield compound **1** (5.3 mg). Fr-5–7 (23.5 mg) was also purified through NP-HPLC using Hexane/EtOAc (75:25) to obtain compound **3** (7.6 mg) and **6** (2 mg). Fr-6 (0.92 g) was subjected to afford 10 sub-fractions through C₁₈ CC eluted with gradient solvent systems of MeOH/H₂O. Fr-6–2 (114 mg), Fr-6–3 (110 mg), and Fr-6–6 (160 mg) were loaded on LH-20 Sephadex CC with isocratic elution of DCM/MeOH (1:1) as a mobile phase, respectively. The resulting semi-pure compounds were further purified by NP-HPLC with Hexane/EtOAc (70:30) to afford compound **5** (2.0 mg), DCM/MeOH (97:3) to provide compounds **2** (10.1 mg), and by RP-HPLC with 35% MeOH to obtain compound **8** (2.0 mg), respectively. Fr-8–2 (450 mg) and Fr-9–2–3 were subjected to further purification using silica gel CC with gradient elution of Hexane/EtOAc and DCM/MeOH, respectively, to give compound **9** (total 4 mg) from both fractions. Finally, Fr-8–6 was purified via NP-HPLC (DCM/MeOH 97/3) to afford compound **10** (3.0 mg).

6 α -hydroxy-3,13E-ene-clerodien-15oic acid (**1**).

A white powder; m. p. in the range of 121–123 °C; UV (MeOH) $\lambda_{\max}$: 249 nm; IR (KBr) $\nu_{\max}$ (cm⁻¹): 3638 (O–H), 2956 (C–H), and 1693 (C=O); CD [nm (mdeg)]: 376 (0.79), 314 (–7.14), 250 (27.37), 230 (–3.45), 208 (15.78); EI-MS m/z 320.3 [M]⁺, HR-EI-MS m/z 320.2344 [M]⁺ (calcd. For C₂₀H₃₂O₃ 320.2351); ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz) see Table 1.

2.4. ECD calculation for compounds 1–4

Conformational analysis was conducted for **1–4** under a molecular mechanics force field (MMFF94) using GMMX software with an energy cutoff of 10 kcal/mol. The density functional theory (DFT) framework with Gaussian 16 was utilized to optimize the obtained conformers. The conformers of compounds **2–4** were optimized using the B3LYP/6–31G (d) level of theory, in the IEFPCM solvent model in MeOH; and conformers of compound **1** were optimized at the B97D/def2-SVP theory

Table 1
¹H NMR and ¹³C NMR data of compound **1**.

No.	δ_{H} (CD ₃ OD, 500 MHz)	δ_{C} (CD ₃ OD, 125 MHz)
1	1.61 (overlapped)	19.0
2	2.02 (overlapped)	27.7
3	5.18 brs	122.7
4		145.6
5		45.2
6	3.44 dd (8.5, 6.5)	76.4
7	1.55 (overlapped)	38.8
8	1.63 (overlapped)	35.9
9		39.6
10	1.30 dd (10.5, 3.5)	47.0
11	1.42 td (12.5, 5.0); 1.53 (overlapped)	37.9
12	2.00 (overlapped); 1.89 td (12.5, 5.0);	35.2
13		159.8
14	5.64 brs	118.2
15		171.6
16	2.10 d (1.0)	19.0
17	0.85 d (6.5)	16.0
18	1.80 brs	22.8
19	1.01 s	15.7
20	0.74 s	18.3

level by IEFPCM solvent model in MeOH. ECD spectrum of **2–4** was obtained at the B3LYP/6–31G(d) level, and **1** was calculated at the B97D/def2-SVP level according to Boltzmann distribution theory [34]. The experimental and calculated spectra were compared using excel.

2.5. Protective effect on glucocorticoid (GC)-treated osteoblasts

2.5.1. Cell culture

MC3T3-E1 cell line was purchased from ATCC (American Type Culture Collection) (CRL-2593, Manassas, VA, USA), and cultured in alpha modified minimum essential medium (α -MEM) (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS) (Thermo Fisher Scientific). Cells were cultured at 37 °C in a humidified atmosphere containing 5% CO₂. A glucocorticoid, dexamethasone (DEX) was used at a concentration of 1 μ M to induce apoptosis in osteoblasts. Geniposide was used as a standard at 10 μ M [27]. Compounds were dissolved in dimethyl sulfoxide (DMSO). Compounds were treated at 0.008, 0.04, 0.2, 1, and 5 μ M concentrations for each experiment.

2.5.2. Cell proliferation assay

Cell proliferation of MC3T3-E1 osteoblasts was determined by using an MTT assay. Briefly, 1×10^3 cells were seeded in a 96-well cell culture plate and incubated for 24 h in 5% CO₂ at 37 °C. Cells were then treated with DEX, DEX + standard, or DEX + compound, and further incubated for 48 h. Then 22 μ L MTT reagents (5 mg/mL in PBS) were added in each well to attain a final concentration of 0.5 mg/mL. The microplate was further incubated for 3 h. After that, the culture medium was removed, and 100 μ L DMSO was added to each well to dissolve the crystal formazan. The absorbance was read at 540 nm by a microplate reader (Spectra Max Plus, Molecular Devices, CA, USA).

2.5.3. Cell apoptosis assay

Results from MTT were further verified by flow cytometry analysis. For this MC3T3-E1 cells (1×10^6 /mL) were seeded in 6 well plates and incubated for 24 h, followed by treatment with DEX, DEX + standard, or DEX + compound. The plates were further incubated for 48 h. After that, the dead and live cells were collected, twice washed with PBS, and centrifuged at 2000 rpm for 10 min at 4 °C. The pellet was stained with 3 μ L of propidium iodide and 3 μ L of Annexin V-FITC in 200 μ L of Annexin-binding buffer. Finally, the plates were subjected to evaluate the number of apoptotic cells by fluorescence-activated cell sorting (FACS) analysis.

2.5.4. Determination of ALP activity

Cellular alkaline phosphatase (ALP) activity was quantified by alkaline phosphatase assay. Briefly, cells were seeded in 24-well plates. Followed by treatment with DEX, DEX + standard, or DEX + compound for 48 h. Next, cells were washed and lysed by using lysing lysis buffer containing paranitrophenylphosphatase (6 mM) and incubated at 37 °C for 1 h. Finally, 30 μ L NaOH (1.5 M) was added to stop the reaction, and absorbance was recorded at 400 nm by using spectrophotometer reader (Spectra Max plus, Molecular Devices, CA, USA).

2.5.5. Alizarin red staining

Following incubation after treatment with DEX, DEX + standard, or DEX + compound, PBS was used to rinse the cell cultures 3 times, and 4% paraformaldehyde (30 min) was used to fix, and then Alizarin Red was used to stain (30 min). The plates were then analyzed under a light microscope (fluorescence microscope TE-2000, Nikon Japan).

2.5.6. Statistical analysis

Statistical analysis was performed by using a one-way analysis of variance, and all data were presented as mean $\pm$ SD.

2.6. Oxidative burst assay

The oxidative burst method, which employs a luminol-enhanced chemiluminescence technique [35], was used. Diluted human blood (25 μ L) in buffer HBSS++ (Helfand Balanced Salt Solution purchased from Sigma-Aldrich, St. Louis, USA) was treated with the compounds or standard ibuprofen (concentrations: 1, 10, and 100 μ g/mL; volume: 25 μ L) each in triplicate. Control wells contained cells and HBSS++ in the absence of compounds. The compounds or the standard were incubated with blood at 37 $^{\circ}$ C for 15 min in 96 well half area plate in the thermostatic luminometric chamber [Labsystems, Finland]. 25 μ L of serum opsonized zymosan (SOZ) obtained from Fluka Switzerland, and 25 μ L of luminol purchased from Research Organics, USA were added, except into blank wells (containing only HBSS++ only). Relative light units (RLU) were used to count the level of reactive oxygen species (ROS) generation.

2.7. Cytotoxicity

MCF-7 (breast cancer), NCI-H460 (lung cancer), Hela (cervical cancer) cell lines, and BJ (normal human fibroblast) cell lines were collected from cell culture biobank (PCMD, ICCBS). DMEM medium was used for MCF-7, Hela, and BJ, and RPMI (ATCC modified medium) was used for NCI-H460. The media was supplemented with 10% FBS, 100 IU/mL of penicillin, and 100 μ g/mL of streptomycin, and kept at 37 $^{\circ}$ C in a 5% CO₂ incubator. The inhibition was investigated by MTT assay. Briefly, 100 μ L/well of cell solutions (10×10^4 cells/mL MCF-7 cells, 5×10^4 cells/mL NCI-H460 cells, 6×10^4 cells/mL Hela cells, or 5×10^4 cells/mL BJ cells), were added into 96-well plate and incubated for 24 h at 37 $^{\circ}$ C, before treatment with positive control doxorubicin and tested compounds 6.25–50 μ M, or negative control DMSO for 48 h. After this, 20 μ L of MTT (5 mg/mL) was added to each well and then incubated for 4 h at 37 $^{\circ}$ C. Then 100 μ L DMSO was added to each well to dissolve the purple formazan crystal. The absorbance was analyzed at 570 nm (for MCF-7, NCI-H460, and Hela) or 550 nm (for BJ) using an ELISA plate reader. The IC₅₀ value of active compounds (inhibition >50% at 50 μ M) was calculated using the EZ-Fit.

3. Results and discussion

The molecular formula of compound **1** (Fig. 1) was established as C₂₀H₃₂O₃ by the HR-EI-MS at m/z 320.2344 [M]⁺ (calc. 320.2351), suggesting the presence of five unsaturation degrees. The UV spectrum showed maximum absorption at 249 nm and the IR spectrum displayed absorption bands at 3638 (O–H), 2956 (C–H), and 1692 (C=O) cm^{−1}. The ¹H NMR data (Table 1) exhibited five methyl signals resonating at δ_H 0.74, 0.85, 1.01, 1.80, and 2.10, along with two olefinic protons at δ_H 5.18 (br s) and 5.64 (br s). The ¹³C NMR and DEPT spectrum indicated the presence of 20 carbons, which were composed of five quaternary carbons, five methines, five methylenes, and five methyls. Above NMR data strongly suggested a skeleton of a diterpene. A comparison of the

NMR data with reported literature indicated that **1** was structurally similar to 3,13*E*-clerodien-15-oic acid [9], except for the presence of an oxymethine proton at δ_H 3.44. The proton signal at δ_H 3.44, which corresponded to the carbon signal at δ_C 76.4 in the HSQC spectrum, was correlated with δ_C 15.7 (C-19), 35.9 (C-8), 38.8 (C-7), 45.2 (C-5), and 145.6 (C-4) in the HMBC spectrum. The methine carbon at δ_C 76.4 was correlated with δ_C 1.01 (H₃–19), 1.55 (H₂–7), and 1.63 (H-8). Likewise, the COSY spectrum showed the linear correlation of δ_H 3.44 (H-6) with δ_H 1.55 (H-7). All the evidences support the location of the hydroxyl group at C-6. Furthermore, the α -orientation of hydroxyl was confirmed by the NOESY correlation between δ_H 3.44 (H-6) and δ_H 1.30 (H–10) (Fig. 2). In addition, the trans form of Δ^{13} was confirmed by the chemical shift of CH₃–16 (δ_H 2.10, d, J = 1.0 Hz), which was in good agreement with the similar compound 3,13*E*-clerodien-15-oic acid [9]. Besides, CH₃–16 (δ_H 2.10) did not show correlation with H-14 (δ_H 5.64 brs) in the NOESY spectrum, which also further proved Δ^{13} as a trans double bond.

Neo-clerodane-type and ent-clerodane-type diterpenes have the same correlations in the NOESY spectrum. Electronic circular dichroism (ECD) calculation is an effective approach to confirming the absolute configuration [36]. After comparison of experimental ECD and calculated ECD of two possible conformations of compound **1** (Fig. 3A), the structure was deduced as ent-clerodane-type, namely 6 α -hydroxy-3,13*E*-ent-clerodien-15-oic acid (**1**) as a new diterpene.

NMR data of compound **2** was identical to 2-oxokolavenic acid [37], which was already isolated from *D. microcarpum* [9–11]. However, the structure was shown as neoclerodane-type by Cavin et al. [11], but ent-clerodane-type by Aquino et al. [10] and Lajide et al. [9], which lead to a confusion about the absolute configuration. Therefore, an ECD calculation was performed for **2**. Finally, compound **2** was determined as ent-clerodane type (Fig. 3B). This is the first time to confirm the absolute configuration of 2-oxokolavenic acid (**2**).

Compound **3** was identified as rhinocerotoic acid, which was reported from *Elytropappus rhinocerotis* [38] and *Plectranthus ornatus* [39]. It was identified from *D. microcarpum* recently through LC-MS/MS by Mbock et al. However, the structure was shown as Δ^{13} Z instead of Δ^{13} E [12], although the NMR data perfectly matched rhinocerotoic acid having Δ^{13} E [38]. Mbock et al. [12] identified the structure based on the reference [40], but in this cited article, the structure was confirmed through another original publication [38]. In this reference [38], Dekker et al. reported the NMR data of both Δ^{13} Z and Δ^{13} E isomers, and the structures were confirmed by X-ray. The chemical shift of CH₃–16 of Δ^{13} E isomer is δ_H 2.20, and of Δ^{13} Z isomer is δ_H 1.98. In the reference of Mbock et al. [12], the chemical shift of CH₃–16 (δ_H 2.14) indicated clearly it is Δ^{13} E form. Therefore, the structure shown in the article (Δ^{13} Z) is wrong. ECD was also performed to confirm the absolute configuration of **3** as labdane-type diterpene (Fig. 3C), which is in agreement with the X-ray structure [38].

Compound **4**'s data was in a good agreement with reported copalic acid from *D. microcarpum* [11]. The relative configuration was confirmed, but the absolute configuration was not determined, and the

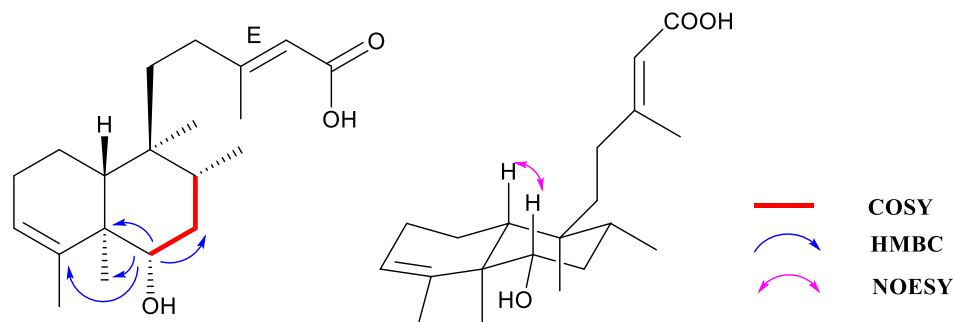


Fig. 2. Key COSY, HMBC, and NOESY correlations of compound **1**.

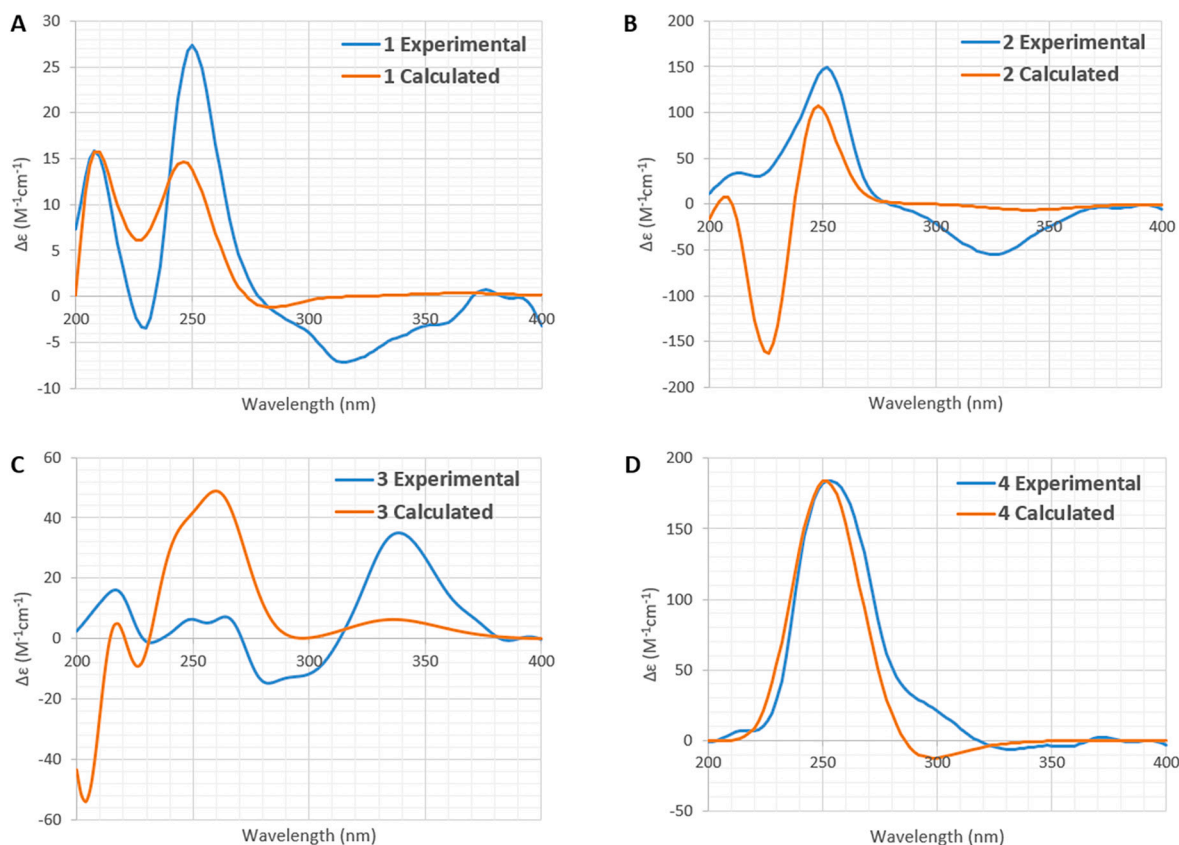


Fig. 3. ECD calculation of compounds 1–4.

structure was drawn as *ent*-labdane type [11]. Therefore, ECD calculation of this compound was also conducted (Fig. 3D), and 4 was demonstrated as a labdane type diterpene. This is the first time to confirm the absolute configuration of copalic acid (4).

The structures of compounds 5–10 were deduced as 6 β -hydroxy-labda-8(17),13*E*-dien-15-oic acid (5) [41], agatholic acid (6) [42], lupeol (7) [4,14,43], daucosterol (8) [4,44], vanillic acid (9) [45], and 4-*O*-methyl gallic acid (10) [43,46] by comparison with reported NMR and Mass data. Among them, compounds 5, 6, 9, and 10 were first time isolated from this plant.

Compounds 1–3 and 8–10 proceeded for protective effect on glucocorticoid (GC)-treated osteoblasts, anti-inflammatory activity, and cytotoxicity evaluation. MTT assay proved that compound 1 possessed a significant protective effect towards dexamethasone (DEX)-induced osteoblast apoptosis at all tested concentrations (0.008, 0.04, 0.2, 1, and 5 μ M). The most potent effective dose was observed at 1 μ M, which is equivalent to the positive control geniposide at 10 μ M (Fig. 4A). The results were also confirmed through flow cytometric analysis. It is observed that DEX (1 μ M) induced apoptosis in osteoblasts by bringing down the osteoblast population to 52.8% in comparison with the non-treated control group having 94.8% cells in the live region. Compound 1 showed a protective effect towards DEX-induced apoptosis in the osteoblasts in a dose-dependent manner between 0.008 and 1 μ M, having the highest cell population of 92.4% at 1 μ M, which was even better than the standard geniposide having 90.1% at 10 μ M (Fig. 4B). Two important markers of osteoblast differentiation and proliferation, alkaline phosphatase (ALP) and mineralization were analyzed. It was observed that alkaline phosphatase levels increased in dose-dependent manner by decreasing the apoptotic effect of DEX, significantly at 1 μ M (Fig. 5A). Alizarin Red staining images showed that DEX significantly induced apoptosis in osteoblasts because less number of cells was observed in images. Compound 1 enhanced the differentiation and proliferation by showing a protective effect at all indicated concentrations. It was

observed that compound 1 at 1 μ M significant stain mineralized nodules and increases the number of cells was also observed which was also near to standard and untreated control (Fig. 5B).

Moreover, compounds 1–3 and 8–10 were also proceeded for anti-inflammatory activity by reactive oxygen species (ROS) production inhibition. Compound 9 (vanillic acid) showed a pronounced inhibition on ROS (IC_{50} 6.5 $\pm$ 0.6 μ M), and 10 (4-*O*-methyl gallic acid) showed a good inhibition with IC_{50} as 103.3 $\pm$ 2.2 μ M, compared with the standard drug ibuprofen (IC_{50} 54.2 $\pm$ 9.2 μ M). This is one more piece of evidence of vanillic acid about its significant anti-inflammatory property, although it is just used as a flavoring agent [47]. Cytotoxicity of compounds 1–3 and 8–10 was evaluated against MCF-7 (breast cancer), NCI-H460 (lung cancer), Hela (cervical cancer), and BJ (normal human fibroblast) cell lines. All tested compounds displayed no cytotoxicity. Compounds 4–7 were not performed for activity evaluation due to limited quantity.

4. Conclusion

This work describes the isolation of a new clerodane diterpene (1) along with nine known compounds (2–10) from *D. microcarpum*. Compound 1 was found to be a potential candidate for the prevention and treatment of osteoporosis with a more significant effect than geniposide. Vanillic acid is further confirmed to have anti-inflammatory potential with a pronounced inhibition of ROS (IC_{50} 6.5 $\pm$ 0.6 μ M) compared with the standard drug ibuprofen (IC_{50} 54.2 $\pm$ 9.2 μ M). Compounds 1–3 and 8–10 were non-cytotoxic on three cancer cell lines and the normal human cell line (BJ).

CRedit authorship contribution statement

Rimsha Irshad: Investigation, Writing – original draft. **Ahmed Saeed Ali Kabbashi:** Investigation. **Kayode Muritala Salawu:**

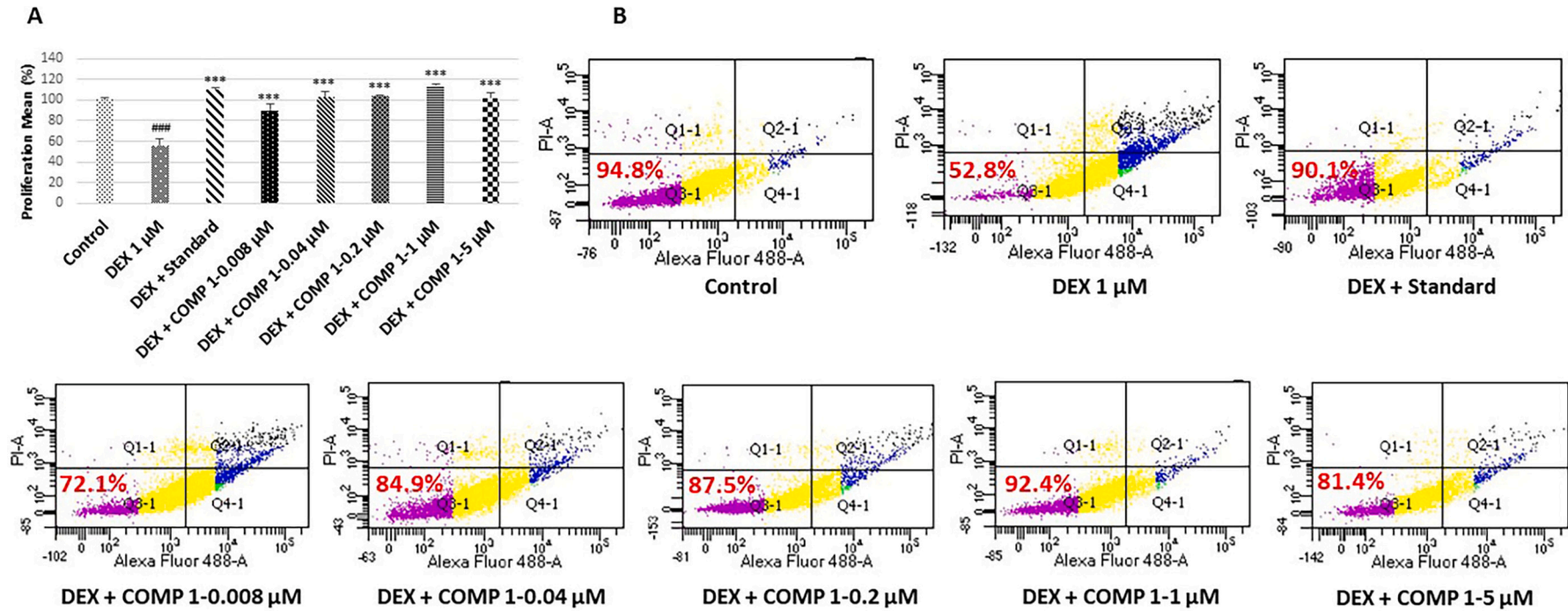


Fig. 4. Effects of compound 1 on the proliferation of dexamethasone (DEX) treated MC3T3-E1 cells. (A) MC3T3-E1 cell proliferation was detected using MTT assays. The data was presented by mean $\pm$ SEM obtained from triple experiments. ### $P < 0.01$ compare with control, *** $P < 0.01$ compare with DEX group. (B) Detection of DEX treated MC3T3-E1 cells survival rate of different groups by flow cytometer. The values in the left bottom quadrant indicate the live cells.

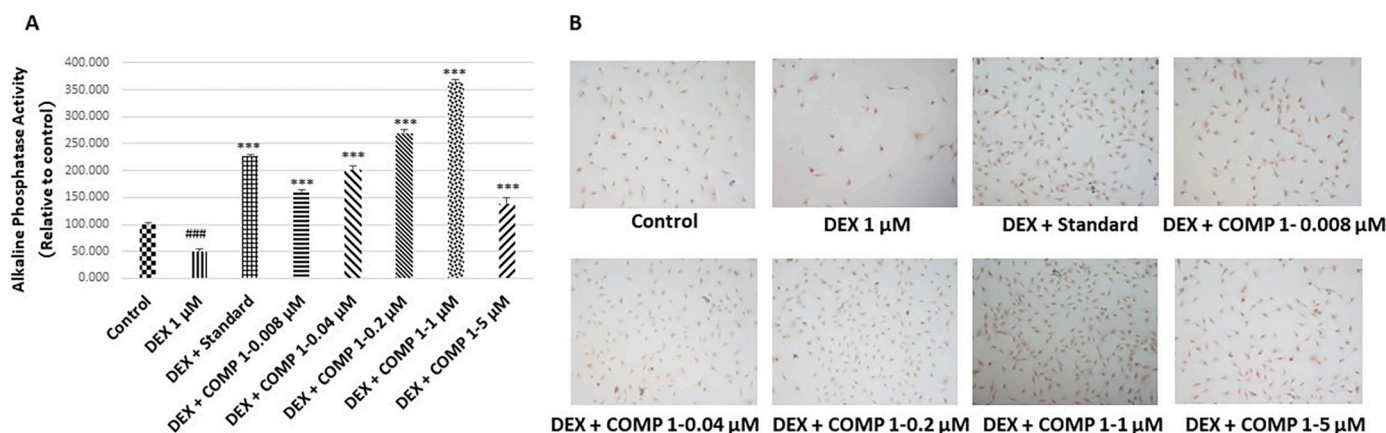


Fig. 5. Quantitative analysis of the expression of alkaline phosphatase (ALP) and Alizarin Red staining. (A) ALP activity in DEX treated MC3T3-E1 cells. The data was presented by mean $\pm$ SEM obtained from triple experiments. ### $P < 0.01$ compare with control, *** $P < 0.01$ compare with DEX group. (B) Alizarin Red staining of the DEX treated MC3T3-E1 cells. Images were captured at $10\times$ magnification. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Resources. Aziz ur-Rehman: Validation. Yan-Gang Cao: Software, Data curation. Aneela Fayaz: Software. Farooq-Ahmad Khan: Writing – review & editing. Atia tul-Wahab: Supervision. M. Iqbal Choudhary: Supervision, Funding acquisition. Yan Wang: Supervision, Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fitote.2022.105226>.

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