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Anti-osteoporotic and antioxidant activities of chemical constituents of the aerial parts of *Ducrosia ismaelis*

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Abstract

A new pterocarpan glycoside, glycinol-3-*O*-β-$_D$-glucopyranoside (**1**), and a new dihydrochalcone glycoside, ismaeloside A (**2**), were isolated together with 13 known compounds, including several flavonoids (**3–8**), lignans (**9-11**), and phenolic compounds (**12-15**), from the methanol extract of the aerial parts of *Ducrosia ismaelis*. The chemical structures of these compounds were elucidated from spectroscopic data and by comparison of these data with previously published results. The anti-osteoporotic and antioxidant activities of the isolated compounds were assessed using tartrate-resistant acid phosphatase (TRAP), oxygen radical absorbance capacity (ORAC), and reducing capacity assays. Compound **15** exhibited a dose-dependent inhibition of osteoclastic TRAP activity with a TRAP value of 86.05 ± 6.55% of the control at a concentration of 10 μM. Compounds **1**, **3-5**, and **8** showed potent peroxyl radical-scavenging capacities with ORAC values of 22.79 ± 0.90, 25.57 ± 0.49, 20.41 ± 0.63, 26.55 ± 0.42, and 24.83 ± 0.12 μM Trolox equivalents (TE) at 10 μM, respectively. Only compound **9** was able to significantly reduce Cu (I) with 23.44 μM TE at a concentration of 10 μM. All of the aforementioned compounds were isolated for the first time from a *Ducrosia* species.

Keywords: *Ducrosia ismaelis* , Apiaceae , Antiosteoporosis, antioxidant, TRAP, ORAC.

Osteoporosis is a chronic, progressive disease of the skeleton characterized by bone fragility due to a reduction in bone mass and possible alterations in bone architecture which lead to a propensity for fractures with minimum trauma.[1] Bone formation is related to osteoblastic proliferation, alkaline phosphatase (ALP) activity, and osteocalcin and collage synthesis. Bone resorption is associated with osteoclast formation and differentiation, and tartrate-resistant acid phosphatase activity (TRAP).[2] Oxidative stress is caused by an imbalance between the generation of reactive oxygen species (ROS) and the activity of antioxidant defence. Severe oxidative stress has been implicated in many chronic and degenerative diseases, including osteoporosis, cancer, ageing, and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.[3] Some natural flavonoids with potent antioxidant activity including scopoletin, reveratrol, and baicalein have found to exert anti-osteoporotic activities through suppressing osteoclast formation and TRAP[4-6]. Accordingly, antioxidants have become a topic of increasing interest.

Ducrosia species belong to the Apiaceae family, the members of which typically contain unusual and/or unique phytochemicals, especially coumarins.[7] In traditional medicine, various *Ducrosia* species are used as analgesics, pain relievers and cold treatments.[8] Antimicrobial, antimycobacterial, antifungal, central nervous system depressant, and antianxiety effects have been reported for several *Ducrosia* species, including *D. anethifolia and D. ismaelis*.[9] *D. ismaelis* Asch. (commonly known as Haza or Geshea) is a perennial, herbaceous, and branched plant with a characteristic aromatic odor.[10] The whole herb, especially its aerial parts, has been used in traditional medicines to treat skin infections and to repel insects and reptiles.[11]

Despite the use of *D. ismaelis* in traditional medicines, there is a lack of information on antioxidant activity which is generally related with prevention of chronic diseases including

osteoporosis and atherosclerosis. In our efforts for the isolation of lead compounds for the treatment of osteoporosis, we investigated the chemical constituents of the *D. ismaelis* plant and evaluated their biological activities. Compounds **1-15** were isolated from the aerial components of *D. ismaelis* (Fig. 1). This report details the isolation, structural determination, and antiosteoporotic and antioxidant activities of these compounds using TRAP, ORAC, and reducing capacity assays. Dried aerial components of *D. ismaelis* were extracted with 100% methanol at room temperature. The crude extract was suspended in water and then successively partitioned with dichloromethane and ethyl acetate to give CH_2Cl_2, EtOAc, and water fractions, respectively. Using various types of column chromatography, two new flavonoid glycosides, named glycinol-3-*O*-β-$_D$-glucopyranoside (**1**)[12] and ismaeloside A (**2**),[13] together with 13 known compounds: daidzin (**3**),[14] daidzein-4'-*O*-β-$_D$-glucopyranoside (**4**),[15] genistin(**5**),[16] prunetrin (**6**),[17] coumestrol (**7**),[18] isobavachalcone (**8**),[19] 4'-hydroxy-3,3',4,5,5'-pentamethoxy-7,9':7',9-diepoxylignane (**9**),[20] liriodendrin (**10**),[21] pinoresinol-4'-*O*-β-$_D$-glucopyranoside (**11**),[22] blumenol C glucoside (**12**),[23] citrusin C (**13**),[24] coniferin (**14**),[25] and (Z)-plicatin B (**15**),[26] were isolated for the first time from *D. ismaelis*.[27] Their chemical structures (Fig.1) were elucidated based on 1D and 2D NMR spectra, MS data, and by comparisons with previously published data acquired from similar compounds (see supplementary information).

Compound **1** was obtained from water fractions as a white amorphous powder (mp 290-295ºC, $[\alpha]_D^{20}$ = -171.31 (*c* 0.1, MeOH), UV λ_{max} (MeOH) nm: 212.0, 283.0, 444.0, 537.0) with a molecular formula of $C_{21}H_{22}O_{10}$, determined by a peak in the MicroQ-TOF III mass spectrum (ESI-Q-TOF MS) at *m/z* 457.1128 $[M + Na]^+$ (calcd for $C_{21}H_{22}O_{10}Na$, 457.1105). FT-IR (KBr) absorbance peak were observed at v_{max} 3350, 2924, 1621, 1499, 1457, 1261, 1171, 1122, 1072, 1037, 967 cm^{-1}. The ^1H NMR spectrum of **1** indicated the presence of an A2-type aliphatic

proton system evidenced by peaks at δ_H 3.83 (1H, d, *J*= 11.4 Hz, H_{ax}-6) and 4.02 (1H, d, *J*= 11.4Hz, H_{aq}-6). Two ABX-type aromatic proton signals also appeared at δ_H 7.29 (1H, *d*, *J*= 8.5 Hz, H-1), 6.71 (1H, dd, *J*= 8.5, 2.3 Hz, H-2) and 6.52 (1H, d, *J*= 2.3 Hz, H-4), due to an A-ring proton, and at δ_H 7.06 (1H, d, *J*= 8.1 Hz, H-7), 6.30 (1H, dd, *J*= 8.1, 2.1 Hz, H-8) and 6.12 (1H, d, *J*= 2.1 Hz, H-10) due to a B-ring proton. A singlet proton peak at δ_H 5.10 (1H, *s*, H-11a), is characteristic of pterocarpans. However, the disappearance of a hydrogen atom and the presence of a hydroxyl group on C-6a (deshielded δ_C 75.83) indicated that **1** is a 6a-hydroxypterocarpan derivative. The ^{1}H NMR spectrum of **1** also contained anomeric proton signal at δ_H 4.80 (1H, d, *J*= 7.4 Hz). The coupling constant of the anomeric proton and its doublet multiplicity confirmed the *β*–configuration of the glucose moiety. The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** indicated 21 carbon atoms including two methylene, 12 tertiary, and seven quaternary carbon atoms. These included one anomeric carbon atom at δ_C 100.66, four oxygenated methine carbon atoms at δ_C 69.96, 73.49, 76.59 and 76.83, and one oxygenated methylene carbon atom at δ_C 61.07 that were assigned to the sugar moiety. The remaining carbon atoms belonged to the aglycone moiety. The β-glucopyranosyl unit was positioned at C-3, which is in agreement with correlations observed between the anomeric proton H-1' (δ_H 4.80) and C-3 (δ_C 158.81) in the HMBC spectra (see Fig. 2). The ^{1}H NMR coupling constant of the anomeric proton was 7.4 Hz with doublet multiplicity, which confirmed the β-configuration of the glucose moiety. The presence of a D-glucose unit was further confirmed by acid hydrolysis and gas chromatographic (GC) analyses followed by comparisons of retention times with authentic samples.[28,29] ^{1}H-^{1}H COSY spectra showed correlations between H-1 (δ_H 7.29) and H-2 (δ_H 6.71), and between H-7 (δ_H 7.06) and H-8 (δ_H 6.30) (see Fig. 2). The absolute configurations were determined as 6a-*S* and 11a-*S* by CD spectral analyses (*c* 0.1, MeOH). A

negative Cotton effect at 234.2 nm and a positive Cotton effect at 286.4 nm were observed.[12] Based on the above data, compound **1** was identified as a new compound, and named glycinol-3-*O*-β-$_D$- glucopyranoside (Fig. **1**).

Compound **2** was obtained from water fractions as a white amorphous powder (mp 130 -133ºC $[\alpha]_D^{20}$ = 21.01 (*c* 0.1, MeOH), UV λ_{max} (MeOH) nm: 224.0, 278.0, 538.0) with a molecular formula of $C_{21}H_{24}O_{10}$, as determined by a peak in the MicroQ-TOF III mass spectrum (ESI-Q-TOF MS) at *m/z* 459.1297 $[M + Na]^+$ (calcd for $C_{21}H_{24}O_{10}Na$, 459.1267). FT-IR (KBr) absorbance peaks were observed at ν_{max} 3345, 2924, 1716, 1613, 1514, 1456, 1288, 1259, 1174, 1073, 1038, 800 cm^{-1}. The ^1H NMR spectrum of **2** contained methylene proton signals appearing at δ_H 3.44 - 3.52 (2H, m, H-β) and an oxymethine proton resonating at δ_H 5.22 (1H, br.s, H-α). ABX-type aromatic proton signals were observed at δ_H 6.41(1H, dd, *J*= 8.3, 2.2 Hz, H-5'), 6.64 (1H, d, *J*= 2.2 Hz, H-3'), and 6.98 (1H, d, *J*= 8.3 Hz, H-6'). AA'BB'-type aromatic proton signals were observed at δ_H 6.79 (2H, d, *J*= 8.4 Hz, H-2,6) and 6.56 (2H, d, *J*= 8.4 Hz, H-3,5). Proton signals corresponding to a β-glucopyranosyl group with an anomeric proton signal at δ_H 4.76 (1H, d, *J*= 7.2 Hz) were also observed. The coupling constant of the anomeric proton and its doublet multiplicity confirmed the β–configuration of the glucose moiety. The ^{13}C NMR and DEPT spectra of **2** indicated 21 carbon atoms, including 2 methylene, 13 tertiary, and 6 quaternary carbon atoms. Among these, a peak corresponding to one anomeric carbon atom at δ_C 102.14, four oxygenated methine carbon atoms at δ_C 70.02, 73.51, 75.25 and 76.51, respectively, and one oxygenated methylene carbon atom at δ_C 61.28 were assigned to the sugar moiety. The remaining carbon atoms were assigned to the aglycone moiety. The β-glucopyranosyl unit was positioned at C-2', which is in agreement with correlations observed between the anomeric proton H-1" (δ_H 4.76) and C-2' (δ_C 155.90) in the HMBC spectra (see Fig. 3). The β-configuration of the glucose

moiety was confirmed by the 7.2-Hz coupling constant of the anomeric proton and by its doublet multiplicity. The presence of a D-glucose unit was further confirmed by acid hydrolysis and gas chromatographic (GC) analyses followed by comparisons of retention times with authentic samples.[28,29] ^{1}H-^{1}H COSY spectra of **2** showed correlations between H-2 (δ_H 6.79) and H-3 (δ_H 6.56), H-5 (δ_H 6.56) and H-6 (δ_H 6.79), and between H-5' (δ_H 6.41) and H-6' (δ_H 6.98) (see Fig. 3). The absolute configuration was determined as α-*R* by CD spectral analysis (*c* 0.1, MeOH) where a positive Cotton effect at 292.4 nm was observed.[30] Based on the above data, compound **2** was determined to be a new compound named ismaeloside A (Fig. **1**).

Compound **15** was identified as (*Z*)-plicatin B based on a comparison of its ^{1}H NMR data with those of (*E*)-plicatin B. The observed coupling constant of 12.0 Hz between H-1' (δ_H 6.76) and H-2' of **15** (δ_H 5.74) (see Table 3) was compared to the 16.0-Hz coupling constant between H-1' and H-2' in (*E*)-plicatin B.[26] The ^{1}H and ^{13}C NMR data of **15** have not been published previously.

The anti-osteoporotic activities of compounds **1-15** were determined using TRAP assays (see supplementary information). TRAP activity was expressed as the percent of the untreated control (mean ± one standard deviation, n = 3). RANKL treatments induced osteoclast formation from RAW 264.7 pre-osteoclast cells and dramatically enhanced TRAP activity up to 170.9%, as shown in Fig. 4. Compounds **15**, **13**, **4**, and **10** suppressed osteoclast formation in a dose-dependent manner with TRAP values of 85.05 ± 6.55, 100.93 ± 6.12, 104.77 ± 11.17, and 106.05 ± 7.65 (% of control) at concentrations of 10 μM, respectively. The potent TRAP activity was observed in compound **3**, **4**, **8**, **10**, **12**, **13**, and **15** at 10 μM when compared to daidzein used as a positive control. Compound **15** exhibited the highest activity of the tested compounds, as indicated by the relatively low TRAP value (Fig. 4). This may be due to the higher cell membrane permeability of **15**, resulting from the absence of a glucose unit and the presence of

two hydrophobic side chains. This agrees with the conclusions of previous work in which scopoletin was more effective than scopolin in terms of reducing the number and TRAP activity of multinucleated TRAP-positive cells in a dose-dependent manner.[4]

According to previous reports, the anti-osteoporotic activities of natural flavonoids as scopoletin, resveratrol, and baicalein are found to be very closely related with their antioxidant activities[3-6]. Thus, the antioxidant activities of compounds **1-15** were investigated in terms of their peroxyl radical-scavenging and reducing capacity. Based on the chemical reactions involved, major antioxidant capacity assays can be roughly divided into hydrogen atom transfer-based and single electron transfer-based assays.[31] ORAC assay is based on hydrogen atom transfer while reducing capacity assay depends upon single electron transfer.[31] Figure 5 shows that the scavenging activities of compounds **1-15** in the presence of peroxyl radicals were dose-dependent at 1−10 μM (see supplementary information). Compounds (**1**, **3**-**5**, and **8**) showed potent peroxyl radical-scavenging activities over 20 TE at 10 μM. In particular, at a concentration of 10 μM, **5** showed the strongest peroxyl radical-scavenging activity with 26.55 ± 0.42 Trolox equivalent (TE) against peroxyl radicals generated from 2,2′ -azobis(2-amidinopropane) dihydrochloride (AAPH). Based on structural analysis of the active isolates (**1-5, 8-9** and **15**), the hydroxyl groups on the aromatic ring seem to be responsible for peroxyl radical-scavenging activity by donating hydrogen atoms to peroxyl radicals.

The ability to transfer single electron was also examined using reducing capacity assay (see supplementary information). The ability of the isolated compounds **1-15** to stimulate the reduction of copper ions (Cu^{2+} to Cu^{+}) by donating electrons was investigated to determine whether their peroxyl radical-scavenging capacities, which involve the donation of hydrogen atoms, could be related to their reduction capacities, which involve donating electrons to copper

ions. As shown in Figure 6, only compound **9** was able to significantly reduce Cu^{2+} ions, with a reduction value of 23.44 ± 0.45 μM TE at a concentration of 10.0 μM. Present data suggest that the ability of compound **9** to donate hydrogens or electron atoms to peroxyl radicals and to convert them into relatively stable compounds may contribute to its peroxyl radical-scavenging capacity. This data agrees with the conclusions of previous studies.[3,4]

When compared both anti-osteoporotic and antioxidant activities of **1-15,** the potent both TRAP and peroxyl radical scavenging activities were observed in **3**, **4**, and **8** among 15 isolated compounds. In addition, there is no correlation between TARP activity and reducing capacity of tested 15 compounds. Therefore, these results imply that compound **3**, **4**, and **8** may augment antiosteoporotic activity by directly scavenging intracellular reactive oxygen species produced during RANKL-mediated osteoclast differentiation through donating hydrogen atom. In other hand, the potent antiosteoporotic activities of compound **10**, **12**, **13,** and **15** not showing strong antioxidant activities *in vitro* may be attributed to their indirect antioxidant activities by activating the endogenous antioxidant defense system. Thus, more research may be needed to determine whether the potent antiosteoporotic activites compound **10**, **12**, **13**, and **15** result from indirect antioxidant activity.

Regarding the structure-activity relationships of the isolated compounds, note that aglycones penetrate cell membranes more readily than do glycosides. For efficient antioxidant activity in cells, compound must be deglycosylated into its aglycone. Further study using an *in vivo* animal model may be required to elucidate the differences in cell-membrane permeability between glycoside and its aglycone.

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27. Dried aerial parts of *D. ismaelis* Asch. (3.0 kg) were extracted with 100% methanol at room temperature three times. After removal of the solvent under reduced pressure, the methanol extract (258 g) was dissolved in 1.0 L of H_2O to form a suspension that was successively partitioned with dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc), to give CH_2Cl_2 (72.87g), EtOAc (8.45g), and aqueous extract (177.0 g), respectively. Aqueous extract was chromatographed on a column of highly porous polymer (Diaion

HP-20) and eluted with H_2O and MeOH, successively to give four fractions (**1a-1d**). Fraction **1a** was then separated by column chromatography over silica gel (70–230 mesh), using CH_2Cl_2/MeOH/H_2O (10:1:0.1 – 1:1:0.1 v/v/v) gradually as eluents, to afford sub-fraction (**2a–2e**). Sub-fraction **2d** was separated by YMC reverse-phase chromatography, using MeOH/H_2O (0.2:1 v/v) as eluent to give compound-**14** (15.0 mg). Fraction **1b** was chromatographed over silica gel, eluting gradually with $CHCl_3$/MeOH/H_2O (10:1:0.1 – 1:1:0.1 v/v/v), to provide five sub-fractions (**3a-3e**). Sub-fraction **3b** was separated by YMC column with MeOH/H_2O (0.3:1 v/v) as eluent to obtain **1** (10.0 mg) and **4** (50.0 mg). Sub-fraction **3c** was separated by YMC column, using MeOH/H_2O (0.3:1 v/v) as eluent, and further purified by column chromatography over silica gel, eluting with CH_2Cl_2/MeOH/H_2O (5:1:0.1 v/v/v) to obtain **2** (10.0 mg), and **10** (12.0 mg). Fraction **1c** was chromatographed over silica gel eluting gradually with $CHCl_3$/MeOH/H_2O (12:1:0.1 – 1:1:0.1 v/v/v), to afford five sub-fractions (**4a–4e**). Sub-fraction **4a** was separated by YMC column, eluting with MeOH/H_2O (0.6:1-0.7:1 v/v), afford **9** (37.0 mg). Sub-fraction **4c** was separated by YMC column, eluting with MeOH/Me_2CO/H_2O (0.3:0.3:1 v/v/v), further by silica gel column with CH_2Cl_2/MeOH/H_2O (10:1:0.1v/v/v) to obtain **6** (3.0 mg), **11** (12.0 mg), and **12** (5.0 mg). Sub-fraction **4d** was separated by YMC column, using MeOH/Me_2CO/H_2O (0.3:0.3:1 v/v/v), and then by column chromatography over silica gel, eluting with CH_2Cl_2/MeOH/H_2O (20:1:0.1 v/v/v) to give **3** (15.0 mg), and **5** (20.0 mg). Compound **13** (20.0 mg) was isolated from sub-fraction **4e** using YMC column eluting with MeOH/Me_2CO/H_2O (0.3:0.3:1 v/v/v), and further purified by silica gel column, using CH_2Cl_2/MeOH/H_2O (10:1:0.1 v/v/v). EtOAc extract was subjected to column chromatography using silica gel (70–230 mesh), eluting with CH_2Cl_2/MeOH/H_2O (20:1:0.2- 5:1:0.1v/v/v) to give five fractions (**5a–5e**). Repeated silica gel column chromatography of fraction **5c** with n-hexane/EtOAc (10:1-3:1 v/v), further by YMC column and eluting with MeOH/H_2O (1:1), and (1.5:1 v/v) to give **7** (10.0 mg), and **8** (10.0 mg), respectively. Finally, CH_2Cl_2 extract was chromatographed over silica gel (70–230 mesh), using n-hexane/EtOAc/MeOH (20:1:0.1 – 1:1:0.1 v/v/v) gradually as eluents, to afford sub-fraction (**6a–6f**). Repeated silica gel column chromatography of fraction **6d** with n-hexane/EtOAc (9:1 v/v), to give **15** (15.0 mg).

28. Acid Hydrolysis and Sugar Identification. Compounds **1** and **2** (2 mg) each was heated in 3 mL of 10% HCl-dioxane (1:1) at 80 °C for 3 h. After the solvent was removed *in vacuo*, and the residue was partitioned between EtOAc and H_2O to give the aglycone (0.95 mg for compounds **1** and **2**) and the sugar (0.63 mg for sugar **1** and 0.62 mg for sugar **2**), respectively. The sugar components in the aqueous layer were analyzed by silica gel TLC by comparison with standard sugars. The solvent system was CH_2Cl_2/MeOH/H_2O (2:1:0.2 v/v/v), and spots were visualized by spraying with 95% EtOH/H_2SO_4/anisaldehyde (9:0.5:0.5 v/v/v), then heated at 180 °C for 5 min. For sugars, the R_f of glucose by TLC was 0.30. The results were confirmed by GC analysis as follows. The aqueous layer was evaporated to dryness using N_2 gas. The residue was dissolved in 0.1 mL of dry pyridine, and then mixed with $_L$-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL). The reaction mixture was heated at 60 °C for 2 h, and 0.1 mL of trimethylsilylimidazole solution was added, followed by heating at 60 °C for 2 h. The dried product was partitioned with n-hexane and H_2O (0.1 mL, each), and the n-hexane layer was analyzed by gas liquid chromatography (GC): Column: column DB-5 (0.25 mm x 30 m); detector μ-ECD, column temp 250 °C, injector temperature 300 °C, detector temperature 280 °C, carrier gas N_2. The absolute configuration of the monosaccharide was confirmed to be $_D$-glucose by comparison of the retention time of the monosaccharide derivative (t_R 14.83 min) with that of authentic sugar derivative samples prepared in the same manner ($_D$-glucose derivative t_R 14.72 min, $_L$-glucose derivative t_R 15.27 min).

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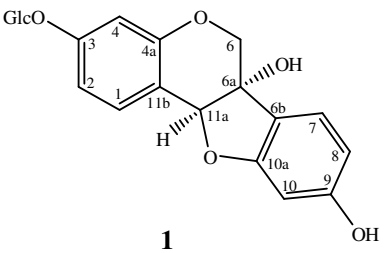

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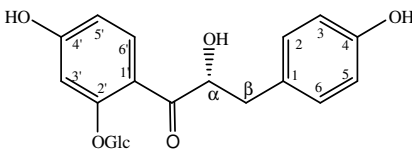

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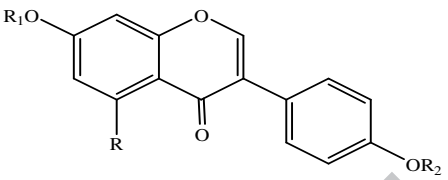
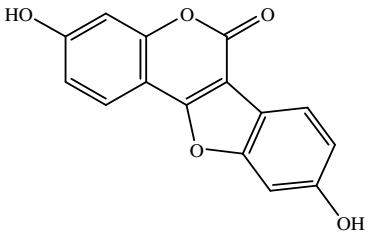


	R	R_1	R_2
3	H	Glc	H
4	H	H	Glc
5	OH	Glc	H
6	OH	Me	Glc



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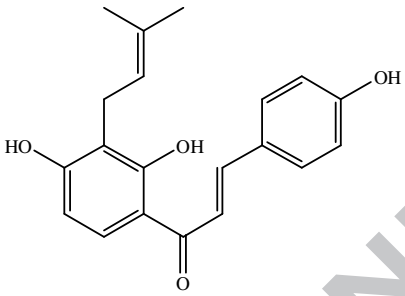

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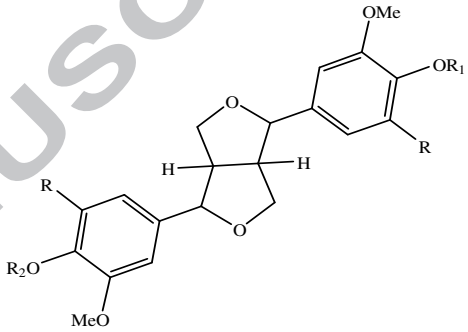
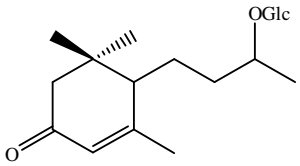


	R	R_1	R_2
9	OMe	H	Me
10	OMe	Glc	Glc
11	H	H	Glc



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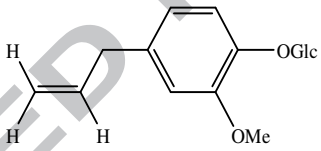

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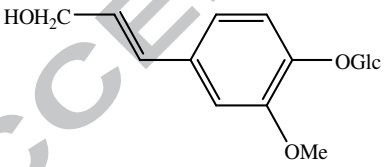

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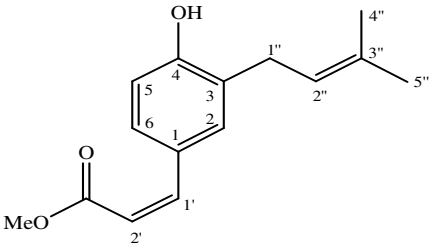

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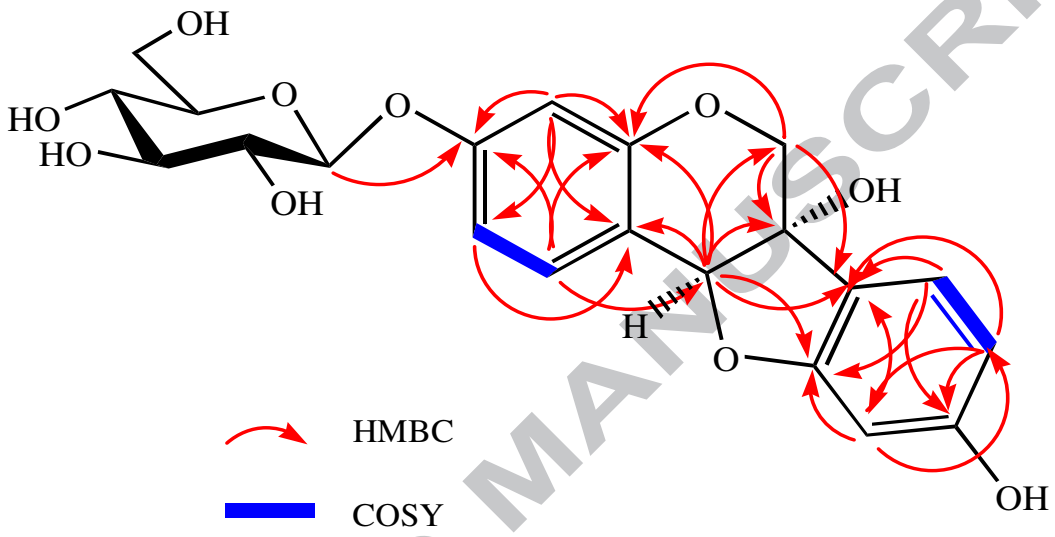
Figure 1. Chemical structures of isolated compounds.



272 **Figure 2.** Selected HMBC and COSY correlation for **1**.

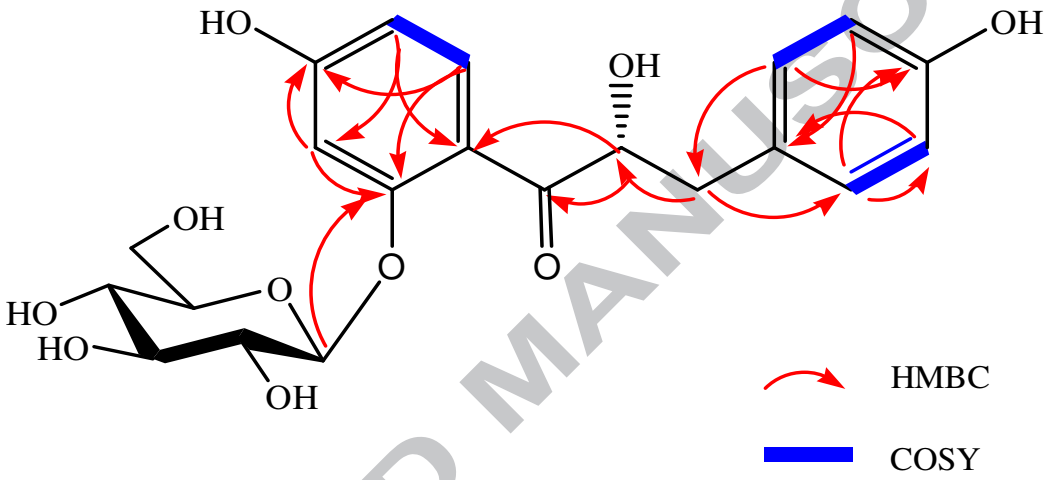
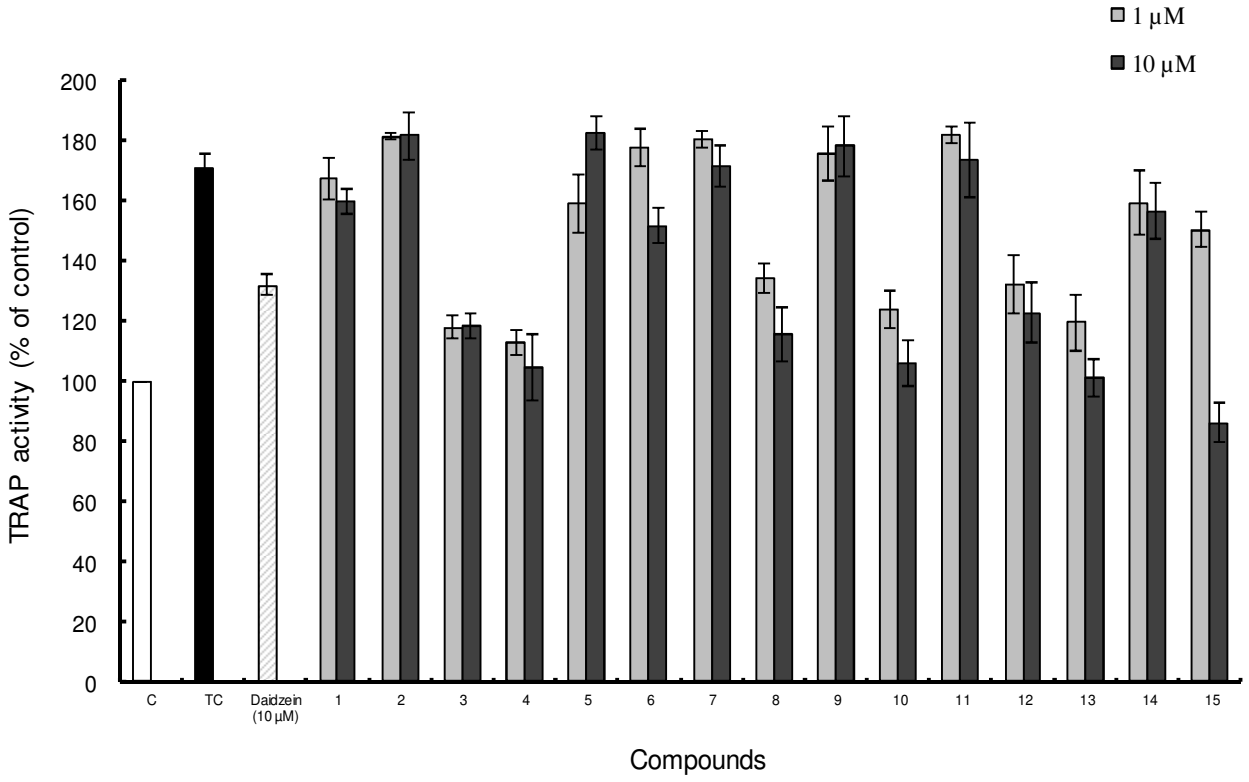


Figure 3. Selected HMBC and COSY correlation for **2**.



274 **Figure 4.** Tartrate-resistant acid phosphatase (% of control) of compounds (**1-15**). C: control,
275 which was not treated; TC: treated control, which was treated with RANKL.

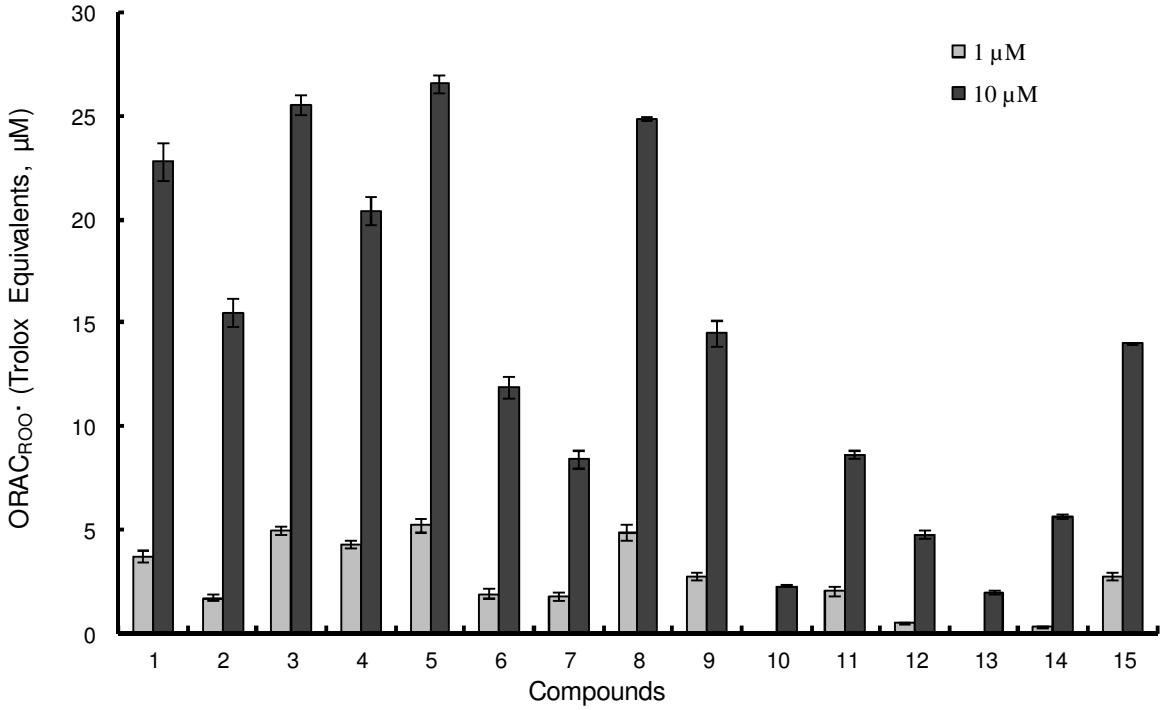
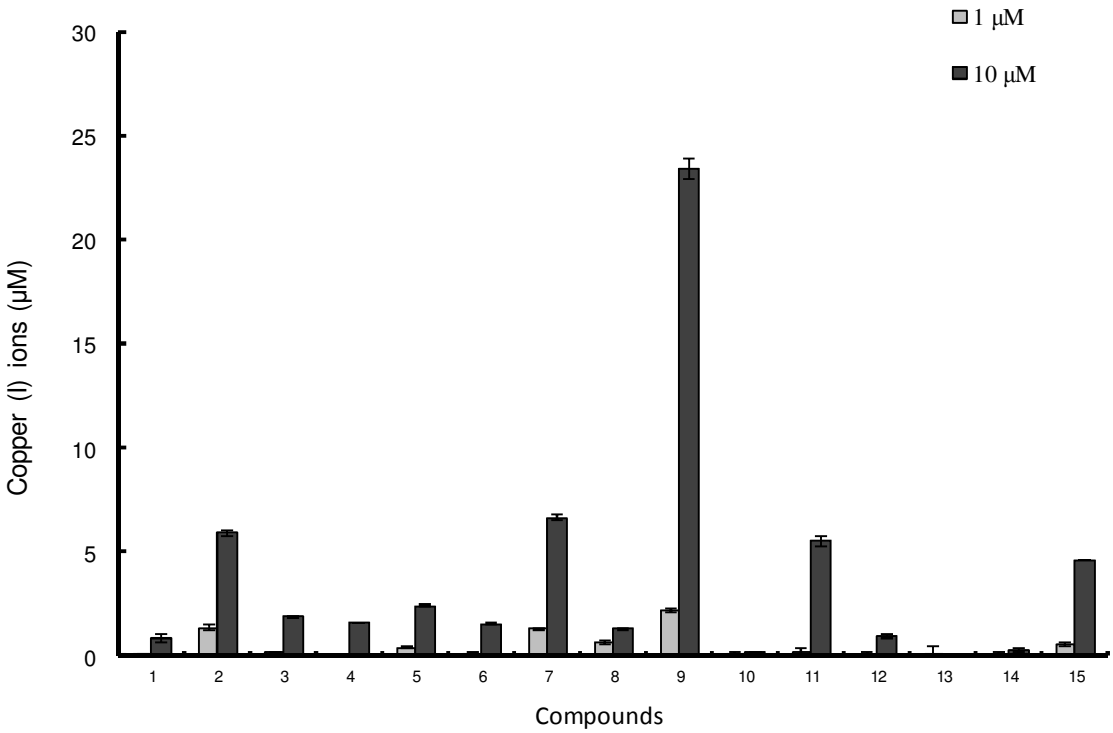


Figure 5. Peroxyl radical-scavenging activity (Trolox Equivalent, μM) of compounds (**1-15**) in ORAC assay.



278 **Figure 6.** Reducing capacities of compounds **1-15**. The results represent the mean ± SD
279 of values obtained from three measurements

Table 1

The ^{1}H and ^{13}C NMR data for **1** in CD_3OD (δ in ppm)

Position	δ_H[a] (H, mult, *J* in Hz)	δ_C[b]
1	7.29 (1H, d, 8.5)	131.79
2	6.71 (1H, dd,8.5, 2.3)	110.72
3		158.81
4	6.52 (1H, d, 2.3)	104.28
4a		155.83
6	3.83 (1H, d, 11.40)	69.57
	4.02 (1H, d, 11.40)	
6a	4.77 (1H, s, OH)	75.83
6b		119.73
7	7.06 (1H, d, 8.1)	123.83
8	6.30 (1H, dd, 8.1, 2.1)	108.06
9		160.81
10	6.12 (1H, d, 2.1)	97.61
10a		159.85
11a	5.10 (1H, s)	84.37
11b		114.69
3-*O*-Glc		
1'	4.80 (1H, d, 7.4)	100.66
2'	3.41- 3.41 (3H, m, 2', 3' and 4')	73.49
3'		76.59
4'		69.96
5'	3.27 (1H, d, 8.9)	76.83
6'	3.56 (1H, dd, 12.1, 1.9)	61.07
	3.75 (1H, dd, 12.1, 1.9)	

Assignments were done by HMQC, HMBC, and ^{1}H–^{1}H COSY experiments.

[a] Measured at 600 MHz.

[b] Measured at 150 MHz.

Table 2

The ^{1}H and ^{13}C NMR data for **2** in CD_3OD (δ in ppm)

Position	δ_H[a] (H, mult, *J* in Hz)	δ_C[b]
C=O		208.63
α	5.22 (1H, br.s)	77.00
β	3.44 – 3.52 (2H, m)	43.58
1		125.08
2 and 6	6.79 (2H, d, 8.4)	130.40
3 and 5	6.56 (2H, d, 8.4)	114.83
4		156.60
1'		119.22
2'		155.90
3'	6.64 (1H, d, 2.2)	103.58
4'		159.27
5'	6.41 (1H,dd, 2.2, 8.3)	109.68
6'	6.98 (1H, d, 8.3)	130.79
2'-*O*-Glc		
1"	4.76 (1H, d, 7.2)	102.14
2"	3.32 – 3.36 (3H, m, H2",3" and 5")	73.51
3"		75.25
4"	3.29 (1H, d, 8.9)	70.02
5"		76.51
6"	3.63 (1H, dd, 6.0, 11.4)	61.28
	3.83 (1H, dd, 1.9, 11.4)	

Assignments were done by HMQC, HMBC, and ^{1}H–^{1}H COSY experiments.

[a] Measured at 600 MHz.

[b] Measured at 150 MHz.

Table 3

The ^{1}H and ^{13}C NMR data for **15** in $CDCl_3$ (δ in ppm)

Position	δ_H[a] (H, mult, J in Hz)	δ_C[b]
C=O		167.27
1		126.60
2	7.48 (1H, dd, 8.4, 2.4)	130.15
3		127.32
4		156.67
5	6.69 (1H, d, 8.4)	115.44
6	7.35 (1H, d, 1.8)	132.66
1'	6.76 (1H, d, 12.0)	143.97
2'	5.74 (1H, d, 12.0)	116.33
1''	3.27 (2H, t, 7.8)	29.78
2''	5.25(1H, m)	121.63
3''		134.92
4''	1.71 (3H, s)	17.96
5''	1.71 (3H, s)	25.88
OCH3	3.65 (3H, s)	51.44
OH	5.69 (1H, br s)	

Assignments were done by HMQC, HMBC, and ^{1}H–^{1}H COSY experiments.

[a] Measured at 600 MHz.

[b] Measured at 150 MHz.

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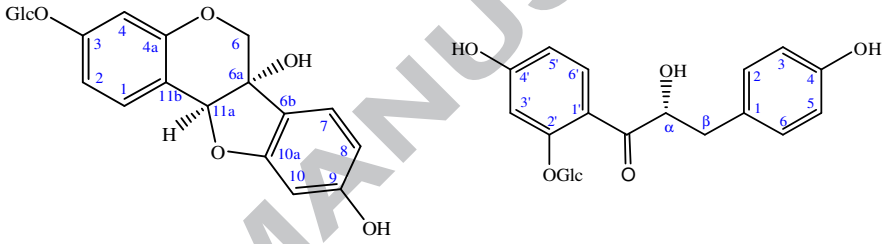
Graphical abstract

Anti-osteoporotic and antioxidant activities of chemical constituents of the aerial parts of *Ducrosia ismaelis*

Abubaker M. Morgan, Hyun Woo Lee, Sang-Hyun Lee, Chi-Hwan Lim, Hae-Dong Jang and Young Ho Kim



1 **2**