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Bioactive Constituents from the Leaves of *Zanthoxylum schinifolium*

Su Yang Jeong, Phi Hung Nguyen, Bing Tian Zhao, Byung Sun Min, Eun Sook Ma, and Mi Hee Woo*

College of Pharmacy, Catholic University of Daegu, Gyeongsan 712-702, Korea

Abstract – Activity-guided separation of the methylene chloride-soluble fraction of the leaves of *Zanthoxylum schinifolium*, resulted in the isolation of four coumarinoids (**1** – **4**), two triterpenoids (**5**, **6**) and three fatty acid derivatives (**7** – **9**) as active principles. Their chemical structures were identified as collinin (**1**), 8-methoxyanisocoumarin (**2**), 7-(6'*R*-hydroxy-3',7'-dimethylocta-2',7'-dienyloxy)-coumarin (**3**), (*E*)-4-methly-6-(coumarin-7'-yloxy) hex-4-enal (**4**), lupeol (**5**), *epi*-lupeol (**6**), phytol (**7**), hexadec-3-enoic acid (**8**) and palmitic acid (**9**), on the basis of spectroscopic (1D, 2D and MS) data analyses and comparing with the data published in the literatures. Compounds **1** and **7** showed potent cytotoxicity against Jurkat T cells with IC_{50} values of 45.58 and 47.51 μM, respectively. The others showed moderate activity with IC_{50} values ranging around 80.58 to 85.83 μM, while the positive control, auraptene, possessed an IC_{50} value of 55.36 μM.
Keywords – *Zanthoxylum schinifolium,* Rutaceae, Cytotoxicity, Collinin, Phytol

Introduction

Zanthoxylum schinifolium (Rutaceae) is an aromatic plant, whose pericarp is widely used as a pungent condiment and seasoning in Korea and in other East Asian countries.[1] The leaves, fruits, seeds and roots of *Z. schinifolium* have been used in the treatment of various diseases, such as inflammation, toothache, muscle pain, and ascarid.[2-4] Medicinal activities have also been reported from this plant, including anti-platelet aggregation, anti-oxidant, inhibition of the production of monoamine oxidase, and anti-tumor.[5-8] Despite many researches on *Z. schinifolium*, our aim is focused on the investigation into cytotoxic components from *Z. schinifolium*. Thus, the roots, stems, pericarps, and seeds of *Z. schinifolium* were extracted using MeOH, while the leaves were extracted using 80% MeOH. These extracts were examined for MTT cytotoxicity against the Jurkat T cell lines, and the result revealed that the leaves extract had a potent MTT cytotoxicity. The leaves extract was subsequently fractionated into four parts: methylene chloride, ethyl acetate, *n*-butanol, and water fractions. The methanol extract and fractions were examined for their cytotoxicity using an *in vitro* MTT assay on Jurkat T cells. Among the samples tested, the methylene chloride fraction showed the strongest inhibition against Jurkat T cell lines. Thus, assay-directed separation of this methylene chloride fraction has led to the isolation of nine active principles, including four coumarinoids (**1** – **4**), two triterpenoids (**5**, **6**), and three fatty acid derivatives (**7** – **9**). In this study, the isolation, structural elucidation, along with the cytotoxicity of the isolates will be discussed herein.

*Author for correspondence
Mi Hee Woo, College of Pharmacy, Catholic University of Daegu, Gyeongbuk 712-702, Korea.
Tel: +82-53-8503620; E-mail: woomh@cu.ac.kr

Experimental

General experimental procedures – Melting points were determined on a Yanaco micro melting point apparatus. Optical rotations were measured on a JASCO DIP-370 digital polarimeter (Japan). IR spectra were measured on a Mattson Polaris FT/IR-300E spectrophotometer. UV spectra were measured on a Thermo 9423 AQA2200E UV spectrophotometer. The NMR spectra were recorded on a Varian Unity INOVA-400 spectrometer (USA), and chemical shifts are expressed as δ value using TMS as an internal standard. Low- and high-resolution EI-MS data were collected on a Quattro II spectrometer. Silica gel 60 (70 - 230 and 230 - 400 mesh, Merck) and Lichroprep RP-18 (40 - 63 μm, Merck) were used as stationary phases for column chromatography. For TLC and HPTLC, silica gel 60 F_{254} (EM 5715, 5628) glass plates (0.25 mm) were used and visualized by spraying with 10% H_2SO_4 and subsequent heating. All other chemicals and solvents were of analytical grade and used without further purification.

Plant material – The leaves of *Z. schinifolium* were collected in Gyeongsan, Gyeongbuk, Republic of Korea in June 2004. These materials were confirmed taxonomically by Professor Chong Won Kim, Catholic University of Daegu, Gyeongsan, Republic of Korea. A voucher specimen has been deposited at the College of Pharmacy, Catholic University of Daegu.

Extraction and isolation – The leaves of *Z. schinifolium* (30 kg) were freeze-dried and powdered, to yield 10 kg of leaf powder. The powder was extracted with 80% MeOH, to yield 1.2 kg of extract, upon removal of the solvent. The MeOH extract was mixed in H_2O (5 L), and the resulting H_2O layer was partitioned with CH_2Cl_2 (4 L × 311 g), *n*-BuOH (4 L × 385 g) and H_2O (300 g). The CH_2Cl_2 extract (311 g) was loaded on a silica gel column (9 × 75 cm, silica-gel 230~400 mesh), and eluted by a stepwise gradient of *n*-hexane-EtOAc (100 : 0 to 1 : 100). The eluates were combined into 23 pools (SL-MC-A~W), on the basis of silica gel TLC. SL-MC-K (3.0 g) was chromatographed on a reverse-phase column (3.5 × 15 cm, RP C-18, and eluted by a stepwise gradient of H_2O-MeOH (40 : 60 to 0 : 100)), to yield compound **1** (500.1 mg). SL-MC-Q (89.3 mg) was chromatographed on a reverse-phase column (3.5 × 15 cm, RP C-18, and eluted by a stepwise gradient of H_2O-MeOH (30 : 70 to 0 : 100), to yield compound **2** (4.2 mg). SL-MC-F (1.6 g) was chromatographed on a silica gel column (3.5 × 15 cm, using the *n*-hexane-CH_2Cl_2 mixture as a solvent, and eluted with a stepwise gradient (100 : 1 to 40 : 1)), to yield compound **6** (400.4 mg). SL-MC-H (2.0 g) was chromatographed on a silica gel column (3.5 × 15 cm, using the *n*-hexane-EtOAc mixture as a solvent, and eluted with a stepwise gradient (100 : 1 to 50 : 1)), to yield compounds **5** (100.2 mg) and **7** (550.1 mg), respectively. SL-MC-I (2.8 g) was chromatographed on a silica gel column (3.5 × 15 cm, using the *n*-hexane-EtOAc mixture as a solvent, and eluted with a stepwise gradient (40 : 1 to 10 : 1)), to yield compounds **3** (19.4 mg), **4** (5.2 mg), and **9** (5.3 mg), respectively. SL-MC-N (1.4 g) was chromatographed on a reverse-phase column (3.5 × 15 cm, RP C-18, and eluted by a stepwise gradient of H_2O-MeOH (40 : 60 to 0 : 100)), to yield compound **8** (42.3 mg).

Collinin (1) – White powder. IR (KBr) cm^{-1}: 2921 (C-H), 1704 (C=O), 1603 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 6.24 (1H, d, *J* = 9.6 Hz, H-3), δ 7.61 (1H, d, *J* = 9.6 Hz, H-4), δ 7.13 (1H, d, *J* = 8.8 Hz, H-5), δ 6.86 (1H, d, *J* = 8.8 Hz, H-6), δ 4.68 (2H, d, *J* = 6.4 Hz, H-1'), δ 5.48 (1H, t, *J* = 6.4 Hz, H-2'), δ 2.13~2.04 (4H, m, H-4',5'), 5.07~5.04 (1H, m, H-6'), δ 1.65 (3H, s, H-8'), δ 1.74 (3H, s, H-9'), δ 1.59 (3H, s, H-10'), δ 3.97 (3H, s, H-OCH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.9 (C-2), δ 113.9 (C-3), δ 143.9 (C-4), δ 113.6 (C-4a), δ 122.9 (C-5), δ 110.4 (C-6), δ 155.2 (C-7), δ 142.0 (C-8), δ 148.4 (C-8a), δ 66.5 (C-1'), δ 119.2 (C-2'), δ 136.9 (C-3'), δ 39.7 (C-4'), δ 26.4 (C-5'), δ 123.9 (C-6'), δ 132.1 (C-7'), δ 25.9 (C-8'), δ 16.9 (C-9'), δ 17.9 (C-10'), δ 61.6 (C-O<u>C</u>H_3); EI-MS *m/z*: 328 $[M]^+$.

8-Methoxyanisocoumarin (2) – Colorless syrup. IR (KBr) cm^{-1}: 3400 (OH), 2960 (C-H), 1725 (C=O), 1610 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 6.25 (1H, d, *J* = 9.6 Hz, H-3), δ 7.64 (1H, d, *J* = 9.6 Hz, H-4), δ 7.37 (1H, d, *J* = 8.4 Hz, H-5), δ 6.86 (1H, dd, *J* = 8.4, 2.4 Hz, H-6), δ 6.82 (1H, d, *J* = 2.4 Hz, H-8), δ 4.61 (2H, d, *J* = 6.4 Hz, H-1'), δ 5.49 (2H, t, *J* = 6.4 Hz, H-2'), δ 2.79 (2H, d, *J* = 6.4 Hz, H-4'), δ 5.65 (2H, m, H-5',6'), δ 1.33 (3H, s, H-8'), δ 1.75 (3H, s, H-9'), δ 1.33 (3H, s, H-10'); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.3 (C-2), δ 113.2 (C-3), δ 143 (C-4), δ 113.4 (C-4a), δ 128.9 (C-5), δ 112.7 (C-6), δ 161.5 (C-7), δ 101.7 (C-8), δ 156.1 (C-8a), δ 65.6 (C-1'), δ 119.5 (C-2'), δ 141.3 (C-3'), δ 42.3 (C-4'), δ 124.0 (C-5'), δ 140.7 (C-6'), δ 70.9 (C-7'), δ 30.0 (C-8'), δ 17.0 (C-9'), δ 30.0 (C-10'); FAB-MS *m/z* : 337 $[M + Na]^+$.

7-(6'*R*-hydroxy-3',7'-dimethylocta-2',7'-dienyloxy)-coumarin (3) – Colorless crystal. IR (KBr) cm^{-1}: 3329 (OH), 1703 (C=O), 1604 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 6.23 (1H, d, *J* = 9.6 Hz, H-3), δ 7.63 (1H, d, *J* = 9.6 Hz, H-4), δ 7.36 (1H, d, *J* = 8.4 Hz, H-5), δ 6.84 (1H, dd, *J* = 8.4, 2.4 Hz, H-3), δ 6.80 (1H, d, *J* = 2.4 Hz, H-8), δ 4.59 (2H, d, *J* = 6.4 Hz, H-1'), δ 5.49 (1H, t, *J* = 6.4 Hz, H-2'), δ 2.13 (2H, m, H-4'), δ 1.67 (2H, m, H-5'), δ 4.05 (1H, t, *J* = 6.4 Hz, H-6'), δ 4.94 (1H, s, H-8'a), δ 4.84 (1H, s, H-8'b), δ 1.73 (3H, s, H-9'), δ 1.77 (3H, s, H-10'); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.3 (C-2), δ 113.2 (C-3), δ 143.7 (C-4), δ 113.4 (C-4a), δ 128.9 (C-5), δ 112.6 (C-6), δ 161.5 (C-7), δ 101.7 (C-8), δ 156.0 (C-8a), δ 65.6 (C-1'), δ 118.8 (C-2'), δ 142.3 (C-3'), δ 35.6 (C-4'), δ 32.9 (C-5'), δ 75.6 (C-6'), δ 147.5 (C-7'), δ 111.4 (C-8'), δ 17.0 (C-9'), δ 17.7 (C-10'); EI-MS *m/z* : 314 $[M]^+$.

(*E*)-4-methly-6-(coumarin-7'-yloxy)hex-4-enal (4) – Colorless crystal. IR (KBr) cm^{-1}: 2922 (C-H), 1727 (C=O), 1610 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 6.25 (1H, d, *J* = 9.6 Hz, H-3), δ 7.64 (1H, d, *J* = 9.6 Hz, H-4), δ 7.37 (1H, d, *J* = 8.4 Hz, H-5), δ 6.84 (1H, dd, *J* = 8.4, 2.4 Hz, H-6), δ 6.80 (1H, d, *J* = 2.4 Hz, H-8), δ 4.60 (2H, d, *J* = 6.4 Hz, H-1'), δ 5.49 (1H, t, *J* = 6.4 Hz, H-2'), δ 2.43 (2H, t, *J* = 7.2 Hz, H-4'), δ 2.62 (2H, td, *J* = 7.2, 1.2 Hz, H-5'), δ 9.80 (1H, t, *J* = 1.2 Hz, H-6'), δ 1.78 (3H, s, H-7'), δ 6.25 (1H, d, *J* = 9.6 Hz, H-3), δ 7.64 (1H, d, *J* = 9.6 Hz, H-4), δ 7.37 (1H, d, *J* = 8.4 Hz, H-5), δ 6.84 (1H, dd, *J* = 8.4, 2.4 Hz, H-6), δ 6.80 (1H, d, *J* = 2.4 Hz, H-8); ^{13}C

NMR ($CDCl_3$, 100 MHz): δ 65.4 (C-1'), δ 119.6 (C-2'), δ 140.4 (C-3'), δ 31.6 (C-4'), δ 41.9 (C-5'), δ 201.8 (C-6'), δ 17.1 (C-7'), δ 162.1 (C-2), δ 113.2 (C-3), δ 143.7 (C-4), δ 113.3 (C-4a), δ 128.9 (C-5), δ 112.7 (C-6), δ 161.5 (C-7), δ 101.7 (C-8), δ 156.0 (C-8a); EI-MS *m/z* : 272 $[M]^+$.

Lupeol (5) – White powder. IR (KBr) cm^{-1}: 3300 (OH), 2943 (C-H), 1637 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 3.20 (1H, dd, *J* = 11.0, 3.8 Hz, H-3), δ 2.38 (1H, m, H-19), δ 0.97 (3H, s, H-23), δ 0.77 (3H, s, H-24), δ 0.83 (3H, s, H-25), δ 1.03 (3H, s, H-26), δ 0.95 (3H, s, H-27), δ 0.79 (3H, s, H-28), δ 4.69 (1H, d, *J* = 2.4 Hz, H-29a), δ 4.57 (1H, d, *J* = 2.4 Hz, H-29b), δ 1.68 (3H,s, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 38.9 (C-1), δ 27.7 (C-2), δ 79.2 (C-3), δ 39.2 (C-4), δ 55.5 (C-5), δ 18.5 (C-6), δ 34.5 (C-7), δ 41.0 (C-8), δ 50.6 (C-9), δ 37.4 (C-10), δ 21.1 (C-11), δ 25.3 (C-12), δ 38.3 (C-13), δ 43.0 (C-14), δ 27.7 (C-15), δ 35.8 (C-16), δ 43.2 (C-17), δ 28.2 (C-18), δ 48.5 (C-19), δ 151.1 (C-20), δ 30 (C-21), δ 40.2 (C-22), δ 28.2 (C-23), δ 16.2 (C-24), δ 16.3 (C-25), δ 15.9 (C-26), δ 14.7 (C-27), δ 18.2 (C-28), δ 19.5 (C-29), δ 109.5 (C-30); EI-MS *m/z* : 426 $[M]^+$.

***epi*-Lupeol (6)** – White powder. IR (KBr) cm^{-1}: 3666 (OH), 2924 (C-H), 1560 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 3.38 (1H, m, H-3), δ 2.38 (1H, m, H-19), δ 0.96 (3H, s, H-23), δ 0.84 (3H, s, H-24), δ 0.83 (3H, s, H-25), δ 1.03 (3H, s, H-26), δ 0.93 (3H, s, H-27), δ 0.79 (3H, s, H-28), δ 4.69 (1H, d, *J* = 2.4 Hz, H-29a), δ 4.56 (1H, d, *J* = 2.4 Hz, H-29b), δ 1.68 (3H, s, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 38.9 (C-1), δ 27.7 (C-2), δ 79.2 (C-3), δ 39.2 (C-4), δ 55.5 (C-5), δ 18.5 (C-6), δ 34.5 (C-7), δ 41.0 (C-8), δ 50.6 (C-9), δ 37.4 (C-10), δ 21.1 (C-11), δ 25.3 (C-12), δ 38.3 (C-13), δ 43.0 (C-14), δ 27.7 (C-15), δ 35.8 (C-16), δ 43.2 (C-17), δ 48.2 (C-18), δ 48.5 (C-19), δ 151.1 (C-20), δ 30.1 (C-21), δ 40.2 (C-22), δ 28.1 (C-23), δ 16.2 (C-24), δ 16.4 (C-25), δ 15.9 (C-26), δ 14.7 (C-27), δ 18.2 (C-28), δ 19.5 (C-29), δ 109.4 (C-30); EI-MS *m/z* : 426 $[M]^+$.

Phytol (7) – Colorless crystal. IR (KBr) cm^{-1}: 3666 (OH), 2987 (C-H), 1653 (C=C); ^1H NMR ($CDCl_3$, 400 MHz): δ 4.15 (2H, d, *J* = 6.8 Hz, H-1), δ 5.41 (2H, t, *J* = 6.8 Hz, H-2), δ 1.99 (1H, t, *J* = 8.0 Hz, H-4), δ 0.86 (3H, d, *J* = 6.4 Hz, H-16), δ 0.85 (3H, d, *J* = 6.4 Hz, H-17), δ 0.87 (3H, d, *J* = 6.4 Hz, H-18), δ 0.88 (3H, d, *J* = 6.4 Hz, H-19), δ 1.67 (3H, s, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 59.6 (C-1), δ 123.3 (C-2), δ 140.4 (C-3), δ 40.1 (C-4), δ 25.3 (C-5), δ 36.8 (C-6), δ 33.0 (C-7), δ 37.6 (C-8), δ 24.7 (C-9), δ 37.5 (C-10), δ 32.9 (C-11), δ 37.5 (C-12), δ 25.0 (C-13), δ 39.8 (C-14), δ 28.2 (C-15), δ 22.8 (C-16), δ 22.9 (C-17), δ 20.0 (C-18), δ 19.9 (C-19), δ 16.4 (C-20); EI-MS *m/z* : 296 $[M]^+$.

Hexadec-3-enoic acid (8) – White powder. IR (KBr) cm^{-1}: 2919 (C-H), 1693 (C=O), 1054 (C-O); ^1H NMR ($CDCl_3$, 400 MHz): δ 2.94 (2H, d, *J* = 5.6 Hz, H-2), δ 5.51 (1H, td, *J* = 12.8, 5.6 Hz, H-3), δ 5.49 (1H, td, *J* = 12.8, 5.6 Hz, H-4), δ 1.99 (2H, dd, *J* = 12.8, 5.6 Hz, H-5), δ 1.33~1.19 (20H, m, H-6~15), δ 0.86 (3H, t, *J* = 6.4 Hz, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.0 (C-1), δ 37.7 (C-2), δ 134.2 (C-3), δ 122.3 (C-4), δ 32.4 (C-5), δ 31.9 (C-6), δ 29.6~29.5 (C-7~14), δ 22.6 (C-15), δ 13.3 (C-16); EI-MS *m/z* : 264 $[M]^+$.

Palmitic acid (9) – White powder. IR (KBr) cm^{-1}: 2935 (C-H), 1699 (C=O), 1054 (C-O); ^1H NMR ($CDCl_3$, 400 MHz): δ 2.36 (2H, t, *J* = 6.86 Hz, H-2), δ 1.63 (2H, m, H-3), δ 1.31~1.26 (24H, m, H-4~15), δ 0.89 (3H, t, *J* = 6.4 Hz, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 179.9 (C-1), δ 34.2 (C-2), δ 24.9 (C-3), δ 29.9~29.3 (C-4~13), δ 32.1 (C-14), δ 22.9 (C-15), δ 14.3 (C-16); EI-MS *m/z* : 256 $[M]^+$.

MTT Assay – The cytotoxic activity of each compound on Jurkat T cells was analyzed by 3-(4,5-dimethylthiazole-2-yl)-2,5-biphenyl tetrazolium bromide (MTT) assay (Park *et al*., 2000). The Jurkat T cells (2.5×10^4 cells/well) were seeded on a 96-well microplate, and were precultured for 36 h. 50 μL of the MTT solution (1.1 mg/mL) was added to each well, and incubated for an additional 4 h. The colored formazan crystal produced from the MTT was dissolved in dimethyl sulfoxide (DMSO). A plate reader was used to measure the optical density (OD) values of the solutions at 540 nm.

Results and Discussion

The nine compounds (**1** – **9**) (Fig. 1) were isolated from the methylene chloride fraction of the leaves of *Z. schinifolium*, by repetitive column chromatography, using silica gel and RP-C18. Compound **1** was obtained as white powder with a molecular weight of m/z 328 $[M]^+$, based on EI-MS data. Compound **1** exhibited UV absorption bands at 202 and 323 nm. The IR spectrum of **1** showed absorption bands at 1701 cm^{-1} (C=O), 2921 (C-H) and 1603 (C=C). The ^1H NMR spectrum of **1** showed the presence of a 7,8-disubstituted coumarin from the characteristic doublets of H-3 (δ_H 6.24, *J* = 9.6 Hz) and H-4 (δ_H 7.61, *J* = 9.6 Hz), H-5 (δ_H 7.13, 1H, d, *J* = 8.8 Hz) and H-6 (δ_H 6.86, 1H, d, *J* = 8.8 Hz). The terpenyl side-chain exhibited signals due to two olefinic protons at δ_H 5.48 (1H, t, *J* = 6.4 Hz, H-2') and 5.05 (1H, m, H-6'), three methylenic protons at δ_H 2.13~2.04 (4H, m, H-4',5') and δ_H 4.68 (2H, d, *J* = 6.4 Hz, H_2-1'), and three vinylic methyl protons at δ_H 1.65 (3H, s, H-8'), δ_H 1.74 (3H, s, H-

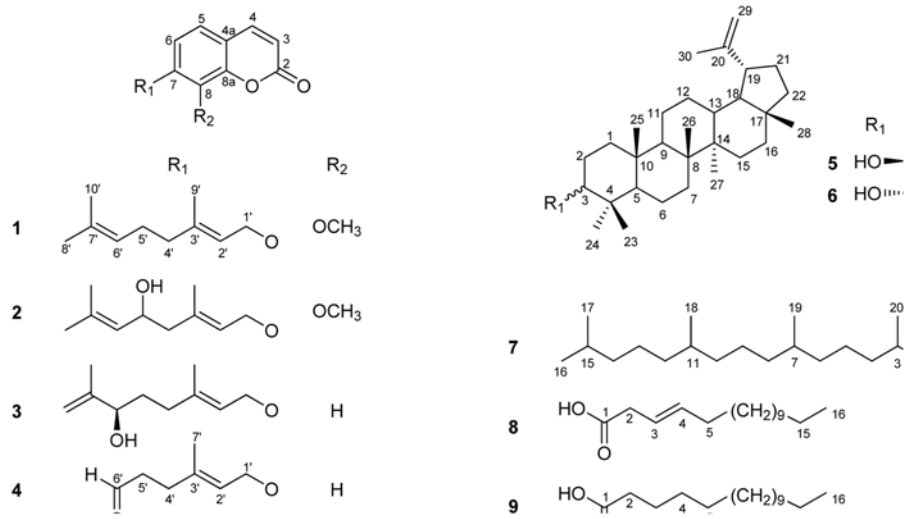

Fig. 1. Chemical structures of compounds **1** – **9** isolated from the leaves of *Z. schinifolium*.

9'), δ_H 1.59 (3H, s, H-10'). The ^{13}C NMR spectrum of **1** displayed all the 20 carbons including nine carbons of the coumarin skeletons at δ_H 160.9 (C-2), 113.9 (C-3), 143.9 (C-4), 113.6 (C-4a), 122.9 (C-5), 110.4 (C-6), 155.2 (C-7) and 142.0 (C-8), 148.4 (C-8a), seven carbons of the terpenyl moiety [δ 66.5 (C-1'), 119.2 (C-2'), 136.9 (C-3'), 39.7 (C-4'), 26.4 (C-5'), 123.9 (C-6'), 132.1 (C-7'), 25.9 (C-8'), 16.9 (C-9'), 17.9 (C-10')] and an additional methoxy unit at δ_C 61.6 (C-O<u>C</u>H$_3$) and δ_H 3.97 (3H, s, H-OCH$_3$). Compound **1** was finally identified as collinin by comparison of the physical and spectroscopic data with those in the literature.[9]

Compound **2** was isolated as colorless syrup, its IR spectrum displayed absorption bands at 3400 cm^{-1} (OH), 2960 (C-H), 1725 (C=O), 1610 (C=C). The molecular weight of compound **2** was obtained from FAB-MS with *m/z* 337 $[M + Na]^+$. The ^1H and ^{13}C NMR spectra of compound **2** were identical with those of compound **1** except only for the signal assignable for C-5' [δ_H 5.65 (1H, m) and δ_C 70.9] and H-4' [δ_H 2.79 (2H, d, J = 6.4 Hz, H-4') and δ_C 42.3], respectively. Compound **2** was therefore elucidated as 8-methoxyanisocoumarin, it was isolated from this plant previously by Tsai *et al*.[10] Compound **3** was obtained as colorless crystal. The IR (KBr) displayed absorption bands at 3329 cm^{-1} (OH), 1703 (C=O), 1604 (C=C). The ^1H and ^{13}C NMR spectra of **3** showed the presence of a 7-substituted coumarin from the characteristic doublets of H-3 (δ_H 6.23, 1H, d, J = 9.6 Hz) and H-4 (δ_H 7.61, 1H, d, J = 9.6 Hz), a pair of ortho-coupled protons of H-5 (δ_H 7.36, 1H, d, J = 8.4 Hz) and H-6 (δ_H 6.86, 1H, d, J = 8.8 Hz) which was meta-coupled with H-8 (δ_H 6.80, 1H, d, J = 2.4 Hz). The terpenyl side-chain exhibited signals due to one olefinic proton at δ_H 5.49 (1H, t, J = 6.4 Hz, H-2'), three methylenic protons at δ_H 4.59 (2H, d, J = 6.4 Hz, H-1'), δ_H 2.13 (2H, m, H-4'), δ_H 1.67 (2H, m, H-5'), and two vinylic methyl protons at δ_H 1.73 (3H, s, H-9'), δ_H 1.77 (3H, s, H-10'). In addition, the ^1H and ^{13}C NMR spectra of **3** possessed an oxygenated olefinic proton at δ_H 4.05 (1H, t, J = 6.4 Hz, H-6') and δ_C 75.6 (C-6') due to the attachment of a hydroxyl group at C-6, an germinal methylene protons at δ_H 4.94 (1H, s, H-8'a), δ_H 4.84 (1H, s, H-8'b) with corresponding carbons at δ_C 147.5 (C-7') and 111.4 (C-8') were further supported for this observation. Detailed comparison of the ^1H and ^{13}C NMR spectra of compound **3** with those published in the literature,[11] led to the structural identification of compound **3** to be 7-(6'*R*-hydroxy-3',7'-dimethylocta-2',7'-dienyloxy)-coumarin.

The ^1H and ^{13}C NMR spectra of compound **4** revealed an identical structure with compound **4**, except for the disappearances of the oxygenated olefinic proton at δ_H 4.05 (1H, t, J = 6.4 Hz, H-6'), the germinal methylene protons at δ_H 4.94 (1H, s, H-8'a), δ_H 4.84 (1H, s, H-8'b) and the vinylic methyl protons at δ_H 1.77 (3H, s, H-10'). Instead of that an aldehyde group was observed in compound **4** at δ_H 9.80 (1H, t, J = 1.2 Hz, H-6') and the corresponding carbon at δ_C 201.8 (C-6'). Compound **4** was thus identified as (*E*)-4-methly-6-(coumarin-7'-yloxy) hex-4-enal.[12] Compounds **5** and **6** were isolated and identified to be lupeol[13] and *epi*-lupeol,[14] respectively by comparing the physicochemical and spectroscopic data with the published literatures. Three fatty acid derivatives were also characterized as phytol (**7**),[15] (**8**) hexadec-3-enoic acid,[16] and (**9**) palmitic acid,[17] respectively. To the best of

Table 1. Cytotoxic activity of isolated compounds **1** – **9** from the leaves of *Z. schinifolium* against Jurkat T cells

Compound	IC_{50} (μM)[a]
1	45.58
2	> 100
3	80.58
4	85.83
5	NT[c]
6	NT[c]
7	47.51
8	> 100
9	96.87
Auraptene[b]	55.36

[a]The inhibitory effects are represented as the molar concentration (μM) giving 50% inhibition (IC_{50}) relative to the vehicle control. These data represent the average values of three repeated experiments.
[b]The compound used as positive control.
[c]The compounds were not tested.

our knowledge, compounds **4**, **6** and **8** were isolated from this plant for the first time.

The nine compounds isolated from *Z. schinifolium* were tested for their cytotoxic activity against the Jurkat T cells,[18] and the result are presented in Table 1. Among those, compounds **1** and **7** showed potential growth inhibition effects with IC_{50} values of 45.58 and 47.51 μM, respectively. While, auraptene, used as the positive control, possessed weaker activity with IC_{50} value of 55.36 μM. Compounds **3**, **4** and **9** showed moderate activity with IC_{50} values ranging around 80.58 to 85.83 μM. The other compounds **8** and **9** were weak (IC_{50} value 96.87 μM) or no active (IC_{50} > 100 μM), respectively. The cytotoxicities of compounds **5** and **6** were not able to investigate due to their insolubility in DMSO.

Compound **1**, collinin, was previously found to be an inhibitor of hepatitis B virus (HBV) DNA replication in a HBV-transfected cell line.[19] Koheno et al. had suggested that certain phenyloxycoumarins, such as auraptene and collinin, could serve as an effective agent against colitis-related colon cancer development in rodents.[20] Costa et al. reported an anticonvulsant effect of phytol in a pilocarpine model in mice.[21] In this study, we found that collinin (**1**) and phytol (**7**) possessed potential cytotoxicity against Jurkat T cells, suggesting that *Z. schinifolium* and its active principles may be useful for the development of agent in combat with cancer.

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