

Prenylated Benzoyltyramine Alkaloids from the Young Fruits of *Naringi crenulata* and Their Cytotoxic Activities

Pichit Sudta^{1*}, Vatcharaporn Prapasanobol¹, Jantana Yahuafai², Siritron Samosorn³, Tawesin Navarat⁴, Teerayut Sriyatep⁴, Yothin Teethaisong⁵, Prayumat Onsrirawat², Jannarin Nontakham², Ananya Bootsaeen⁵

¹Division of Chemistry, Faculty of Science and Technology, Phetchaburi Rajabhat University, Phetchaburi 76000, Thailand.

²Clinical Research and Innovation Section, Division of Medical Research and Technology, Assessment, National Cancer Institute, Bangkok 10400, Thailand.

³Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Srinakharinwirot University, Bangkok 10110, Thailand.

⁴Microbial Biotechnology and Utilization of Natural Products Research Unit, Faculty of Science and Technology, Songkhla Rajabhat University, Songkhla 90000, Thailand.

⁵Department of Medical Sciences, Faculty of Allied Health Sciences, Burapha University, Chon Buri 20131, Thailand.

ARTICLE INFO

Article history:

Received

Revised

Accepted

Published online 01 October 2026

ABSTRACT

The chemical constituents and biological activities of the young fruits of *Naringi crenulata* (Roxb.) Nicolson (Rutaceae) remain poorly investigated. This study aimed to isolate secondary metabolites from the young fruits and evaluate the in vitro cytotoxicity and relative selectivity of the extracts and the isolated compounds. The plant material was sequentially extracted with hexane, CH₂Cl₂, acetone, and MeOH, and cytotoxicity was evaluated against KB, MCF-7, and Vero cells using the MTT assay. Of these extracts, CH₂Cl₂ was the most active against KB and MCF-7 cells, with IC₅₀ values of 15.08 and 27.92 µg/mL, respectively, and showed lower cytotoxicity toward Vero cells (IC₅₀ = 169.65 µg/mL), corresponding to selectivity indices (SI) of 11.25 and 6.07. Three known prenylated benzoyltyramine alkaloids, acidissiminol (1), acidissimin epoxide (2), and acidissiminol (3), were isolated. Their structures were assigned from spectroscopic data acquired in this study and compared with published values. Compound 3 was the most active against KB and MCF-7 cells (IC₅₀ = 0.79 and 0.87 µg/mL; SI = 6.10 and 5.54) but also showed appreciable cytotoxicity toward Vero cells (IC₅₀ = 4.82 µg/mL). Compound 2 was selectively cytotoxic toward KB cells (IC₅₀ = 6.11 µg/mL; SI >16.37), whereas compound 1 was less active. This is the first report of compounds 1–3 from *N. crenulata* and their preliminary evaluation in these cell models.

Copyright: © 2026 Sudta *et al.* This is an open-access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Keywords: *Naringi crenulata*, Prenylated Benzoyltyramine Alkaloids, Cytotoxicity, Selectivity

Introduction

Naringi crenulata (Roxb.) Nicolson (syn. *Hesperethusa crenulata* (Roxb.) M. Roem, *Limonia crenulata* Roxb.), a medicinal tree belonging to the family Rutaceae, is widely distributed across South and Southeast Asia, including Sri Lanka, India, Pakistan, Malaysia, Myanmar, Laos, and Thailand.¹ The plant is known locally as Krakae in Thailand, Thanaka in Myanmar, and Narinarakam in India.^{2,3} Various parts of *N. crenulata* have long been used in traditional medicine. The roots are used to treat vomiting, colic, body pain, and dysentery,^{4,5} whereas the powdered stem is traditionally applied as a facial cosmetic and is reported to possess anti-acne and anti-aging properties.⁶⁻⁸ The bark juice is used as a traditional remedy for puerperal fever and sprains,^{3,9} while the leaves are used to manage dysentery and epilepsy.^{9,10} In addition, a decoction prepared from the fruits is traditionally used as an insect repellent.¹¹

*Corresponding author. Email: pichit.sud@mail.pbru.ac.th
Tel: +66 81773 5018

Citation: Sudta P, Prapasanobol V, Yahuafai J, Samosorn S, Navarat T, Sriyatep T, Teethaisong Y, Onsrirawat P, Nontakham J, Bootsaeen A. Prenylated benzoyltyramine alkaloids from the young fruits of *Naringi crenulata* and their cytotoxic activities. Trop J Nat Prod Res. 2026;10(9).

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Phytochemical investigations of *N. crenulata* have identified diverse classes of secondary metabolites, including alkaloids, coumarins, flavonoids, lignans, quinones, steroids, and terpenoids, particularly from the stems, stem bark, and leaves.¹²⁻¹⁷ Although *N*-benzoyltyramine methyl ether has previously been identified from this species,¹² prenylated benzoyltyramine alkaloids have not, to the best of our knowledge, been reported from *N. crenulata*. These alkaloids are characterized by a benzoyltyramine scaffold bearing an *O*-linked modified prenyl side chain. Structurally related prenylated benzoyltyramine alkaloids isolated from the peels of *Atalantia monophylla* have exhibited cytotoxicity against cholangiocarcinoma, HeLa, HCT116, and MCF-7 cell lines.^{18,19} More recently, severeine palmitate and acidissiminol epoxide from *A. monophylla* were reported to inhibit several human cancer cell lines, with acidissiminol epoxide inducing apoptosis and G0/G1 cell-cycle arrest in MCF-7 cells.²⁰ Nevertheless, prenylated benzoyltyramine alkaloids from *N. crenulata* and their cytotoxic selectivity have not been previously investigated. Accordingly, the novelty of the present study lies in the first reported occurrence of the known compounds 1–3 in *N. crenulata*, together with the evaluation of their cytotoxicity and relative selectivity in the tested cell models.

Cancer remains a major global health concern,²¹ and the clinical effectiveness of many chemotherapeutic agents is often limited by systemic toxicity, damage to healthy tissues, and the development of drug resistance.^{22,23} Consequently, identifying cytotoxic agents that preferentially affect cancer cells while exhibiting lower toxicity toward non-cancerous cells remains an important objective in anticancer drug discovery. Natural products and their structurally diverse secondary

metabolites have long served as valuable sources of lead compounds for the development of anticancer therapeutics.^{24,25} Medicinal plants may therefore provide structurally distinctive metabolites with potentially useful cytotoxic and selective properties.

Based on these considerations, the present study investigated the chemical constituents of the young fruits of *N. crenulata* and evaluated the cytotoxicity of the plant extracts and isolated prenylated benzoyltyramine alkaloids against KB and MCF-7 cancer cells. Their effects on non-cancerous Vero cells were also determined to assess selectivity toward the tested cancer cells.

Materials and Methods

General experimental procedures

¹H and ¹³C NMR spectra were recorded on a Bruker AVANCE 300 FT-NMR spectrometer (Bruker, Germany) at 300 and 75 MHz, respectively, using CDCl₃ as the solvent. The chemical shifts (δ) are reported in ppm with reference to the residual solvent signal of CDCl₃ (δ _H 7.26 and δ _C 77.16), and coupling constants (J) are given in Hz. DEPT, ¹H–¹H COSY, HSQC, and HMBC experiments were performed as appropriate to support the structural assignments.

The purity of compounds **1–3** was assessed qualitatively from their ¹H and ¹³C NMR spectra, which showed no prominent signals attributable to major impurities. High-resolution electrospray ionization mass spectra (HR-ESIMS) were acquired on a compact quadrupole time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany) operating in the positive mode. IR spectra were recorded on a Bruker INVENIO-S FT-IR spectrometer (Bruker, Switzerland). Melting points were determined using a Griffin melting point apparatus (Fisher Scientific, UK) and are uncorrected. Column chromatography was performed using Merck silica gel 60 (<0.063 mm). Analytical TLC was carried out on precoated silica gel 60 F₂₅₄ plates (Merck, Germany). Compounds were visualized under UV light or by spraying with anisaldehyde–H₂SO₄ reagent, followed by heating. Solvents used for extraction and preparative chromatography were of commercial grade and distilled before use.

Plant material

Young fruits of *Naringi crenulata* (Roxb.) Nicolson were collected in June 2025, approximately one month after fruit set, from the Faculty of Agricultural Technology, Phetchaburi Rajabhat University, Phetchaburi, Thailand (13.0746° N, 99.9786° E). The plant material was morphologically identified and authenticated by Bancha Yingngam, PhD, Subdepartment of Pharmaceutical Botany and Pharmacognosy, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand, based on morphological and taxonomic characteristics and comparison with an authenticated herbarium specimen of *N. crenulata* (PHUBU00466) deposited at the Faculty of Pharmaceutical Sciences, Ubon Ratchathani University. A representative voucher specimen of the plant material used in this study was deposited in the Herbarium, Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Thailand, under voucher number PHUBU-NC-2025-001. A duplicate voucher specimen was also deposited in the institutional botanical collection of the Division of Chemistry, Faculty of Science and Technology, Phetchaburi Rajabhat University, Thailand, under voucher number PBRU-005.

Extraction and isolation

The dried and ground young fruits (1.2 kg) of *N. crenulata* were successively extracted by maceration in hexane (4 × 7 L), CH₂Cl₂ (5 × 7 L), acetone (5 × 7 L), and MeOH (4 × 7 L), each for 4 days. After the removal of the solvent using a rotary evaporator under reduced pressure, hexane (green sticky solid, 36.76 g), CH₂Cl₂ (pale green sticky solid, 9.52 g), acetone (dark brown sticky solid, 7.09 g), and MeOH (dark brown sticky solid, 68.34 g) extracts were obtained. The CH₂Cl₂ extract, which exhibited strong cytotoxic activity against KB (IC₅₀ = 15.08 ± 0.51 µg/mL) and MCF-7 (IC₅₀ = 27.92 ± 6.19 µg/mL) cell lines, was selected for further investigation. The CH₂Cl₂ crude extract (5.00 g) was fractionated by CC (Silica gel, eluting with a gradient system of hexane, hexane: CH₂Cl₂ (100:0 to 0:100, v/v), and CH₂Cl₂: acetone (100:0 to 0:100, v/v)) to give five fractions, F1–F5. Fraction F2 (920.6 mg) was

selected for further purification because it contained a relatively large amount of material and showed a distinct major spot on TLC. Fraction F2 was purified by silica gel column chromatography eluting with a gradient system of hexane, hexane: CH₂Cl₂ (100:0 to 0:100, v/v), and CH₂Cl₂: acetone (100:0 to 0:100, v/v) to obtain four subfractions, F2.1–F2.4. Subfraction F2.1 was purified by flash column chromatography using 5% CH₂Cl₂ in hexane (v/v) to afford three subfractions, F2.1.1–F2.1.3. Subfraction F2.1.1 (184.1 mg) was reprecipitated from 30% CH₂Cl₂ in hexane (v/v) to afford acidissimin (**1**, 159.7 mg). Subfraction F2.1.2 (127.8 mg) was fractionated by silica gel column chromatography using a gradient system of hexane, hexane: CH₂Cl₂ (100:0 to 0:100, v/v), and CH₂Cl₂: acetone (100:0 to 0:100, v/v) to give acidissimin epoxide (**2**, 52.7 mg). Subfraction F2.1.3 (80.7 mg) was further purified by silica gel column chromatography using a gradient system of hexane, hexane: CH₂Cl₂, and CH₂Cl₂: acetone to obtain acidissiminol (**3**, 36.3 mg).

Characteristics of the isolated compounds

Acidissimin (**1**). White solid; mp 64–66 °C (lit. 65 °C)²⁶; IR (neat) ν_{max} 3348, 2916, 2848, 1731, 1639, 1530, 1237, 1171, 945, 717 cm^{–1}; ¹H NMR (300 MHz, CDCl₃) δ : 0.87 (3H, t, J = 5.9 Hz, H-18'), 1.25 (28H, m, H-4'-H-17'), 1.60 (3H, s, H-8), 1.63 (2H, m, H-3'), 1.66 (3H, s, H-9), 1.72 (3H, s, H-10), 2.28 (2H, t, J = 7.3 Hz, H-2'), 2.35 (2H, m, H-5), 2.86 (2H, t, J = 6.8 Hz, H-7''), 3.67 (2H, q, J = 6.8 Hz, H-8''), 4.55 (2H, d, J = 6.0 Hz, H-1), 5.00 (1H, t, J = 5.8 Hz, H-6), 5.15 (1H, t, J = 6.7 Hz, H-4), 5.71 (1H, t, J = 6.0 Hz, H-2), 6.18 (1H, br s, N-H), 6.84 (2H, d, J = 8.6 Hz, H-2'' and H-6''), 7.13 (2H, d, J = 8.6 Hz, H-3'' and H-5''), 7.39 (2H, dd, J = 1.4 and 6.8 Hz, H-3''' and H-5'''), 7.47 (1H, dd, J = 1.4 and 6.8 Hz, H-4'''), 7.69 (2H, dd, J = 1.4 and 6.8 Hz, H-2''' and H-6'''); ¹³C NMR (75 MHz, CDCl₃) δ : 13.1 (C-10), 14.1 (C-18'), 17.9 (C-8), 22.6 (C-17'), 25.0 (C-3'), 25.7 (C-9), 29.1–29.7 (C-4'–C-15'), 31.6 (C-16'), 34.6 (C-5), 34.8 (C-2' and C-7''), 41.3 (C-8''), 64.5 (C-1'), 77.5 (C-4), 115.0 (C-2'' and C-6''), 118.9 (C-6), 122.7 (C-2), 126.8 (C-2''' and C-6'''), 128.5 (C-3''' and C-5'''), 129.7 (C-3'' and C-5''), 131.0 (C-4''), 131.3 (C-4'''), 134.7 (C-7 and C-1''), 137.7 (C-3), 157.4 (C-1'), 167.4 (C-9''), 173.0 (C-1'); HR-ESIMS m/z 682.4782 [M + Na]⁺ (calcd for C₄₃H₆₅NNaO₄, 682.4805).

Acidissimin epoxide (**2**). White solid; mp 105–107 °C (lit. 105–106 °C)²⁷; IR (neat) ν_{max} 3345, 2918, 2849, 1729, 1639, 1530, 1348, 1235, 1106, 1026, 977, 719 cm^{–1}; ¹H NMR (300 MHz, CDCl₃) δ : 0.87 (3H, t, J = 6.3 Hz, H-18'), 1.25 (28H, m, H-4'-H-17'), 1.27 (6H, s, H-8 and H-9), 1.63 (2H, m, H-3'), 1.75 (3H, s, H-10), 1.91 (2H, m, H-5), 2.32 (2H, t, J = 7.3 Hz, H-2'), 2.70 (1H, t, J = 5.9 Hz, H-6), 2.87 (2H, t, J = 6.7 Hz, H-7''), 3.51 (2H, q, J = 6.7 Hz, H-8''), 4.56 (2H, d, J = 6.0 Hz, H-1), 5.36 (1H, t, J = 6.4 Hz, H-4), 5.78 (1H, t, J = 6.0 Hz, H-2), 6.16 (1H, br s, N-H), 6.84 (2H, d, J = 8.5 Hz, H-2'' and H-6''), 7.13 (2H, d, J = 8.5 Hz, H-3'' and H-5''), 7.45 (2H, dd, J = 1.4 and 6.9 Hz, H-3''' and H-5'''), 7.49 (1H, dd, J = 1.4 and 6.9 Hz, H-4'''), 7.69 (2H, dd, J = 1.4 and 6.9 Hz, H-2''' and H-6'''); ¹³C NMR (75 MHz, CDCl₃) δ : 13.1 (C-10), 14.1 (C-18'), 18.8 (C-8), 22.7 (C-17'), 24.6 (C-9), 24.9 (C-3'), 29.1–29.7 (C-4'–C-15'), 31.9 (C-16'), 32.6 (C-5), 34.5 (C-2'), 34.8 (C-7''), 41.3 (C-8''), 57.8 (C-7), 60.7 (C-6), 64.3 (C-1), 75.4 (C-4), 114.9 (C-2'' and C-6''), 122.8 (C-2), 126.8 (C-2''' and C-6'''), 128.5 (C-3''' and C-5'''), 129.7 (C-3'' and C-5''), 131.3 (C-4''), 131.3 (C-4'''), 134.7 (C-1''), 137.3 (C-3), 157.3 (C-1'), 167.4 (C-9''), 172.8 (C-1'); HR-ESIMS m/z 698.4733 [M + Na]⁺ (calcd for C₄₃H₆₅NNaO₅, 698.4754).

Acidissiminol (**3**). White solid; mp 86–88 °C (lit. 85–87 °C)²⁷; IR (neat) ν_{max} 3330 (broad), 2966, 2862, 1635, 1577, 1542, 1304, 1242, 1055, 695, 671 cm^{–1}; ¹H NMR (300 MHz, CDCl₃) δ : 1.64 (3H, s, H-8), 1.67 (3H, s, H-9), 1.72 (3H, s, H-10), 2.28 (2H, t, J = 6.9 Hz, H-5), 2.87 (2H, t, J = 6.8 Hz, H-7''), 3.68 (2H, q, J = 6.8 Hz, H-8''), 4.06 (1H, t, J = 6.9 Hz, H-4), 4.58 (2H, d, J = 6.2 Hz, H-1), 5.10 (1H, t, J = 6.9 Hz, H-6), 5.57 (1H, t, J = 6.2 Hz, H-2), 6.15 (1H, br s, N-H), 6.87 (2H, d, J = 8.6 Hz, H-2'' and H-6''), 7.15 (2H, d, J = 8.6 Hz, H-3'' and H-5''), 7.40 (2H, dd, J = 1.6 and 6.5 Hz, H-3''' and H-5'''), 7.46 (1H, dd, J = 1.6 and 6.5 Hz, H-4'''), 7.69 (2H, dd, J = 1.6 and 6.5 Hz, H-2''' and H-6'''); ¹³C NMR (75 MHz, CDCl₃) δ : 12.5 (C-10), 18.0 (C-8), 25.9 (C-9), 34.1 (C-5), 34.8 (C-7''), 41.2 (C-8''), 64.2 (C-1), 76.2 (C-4), 114.9 (C-2'' and C-6''), 119.6 (C-6), 120.9 (C-2), 126.8 (C-2''' and C-6'''), 128.5 (C-3''' and C-5'''), 129.7 (C-3'' and C-5''), 130.9 (C-4''), 131.3 (C-4'''), 134.7

(C-7), 135.3 (C-1'''), 141.7 (C-3), 157.5 (C-1''), 167.4 (C-9''); HR-ESIMS m/z 416.2197 $[M + Na]^+$ (calcd for $C_{25}H_{31}NNaO_3$, 416.2196).

Cytotoxic activity assay

The cytotoxic effects of the extracts and isolated compounds were evaluated using the MTT assay. Human epithelial KB cells (ATCC CRL-3596), human breast adenocarcinoma MCF-7 cells (ATCC HTB-22), and African green monkey kidney Vero cells (ATCC CCL-81) were obtained from the American Type Culture Collection (ATCC, USA). The cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. The cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Cells were seeded into 96-well plates at a density of 3×10^3 cells/well and allowed to attach for 24 h. The cells were subsequently exposed to the extracts (1–1000 µg/mL) or isolated compounds (0.1–100 µg/mL) for 72 h. Doxorubicin (0.01–10 µg/mL) was included as the positive control, while medium containing 0.1% DMSO served as the vehicle control. After the treatment period, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well, followed by incubation for an additional 3 h at 37 °C under 5% CO₂. The medium was then carefully aspirated, and the resulting formazan crystals were dissolved in 100 µL of DMSO. Absorbance was measured at 550 nm using a microplate reader (Benchmark 550, Bio-Rad, USA). Each concentration was tested in six replicate wells, and three independent experiments were conducted. Cell viability was expressed as a percentage relative to the vehicle-treated control. The IC₅₀ value was estimated by interpolation from the dose–response curve of percentage cell viability versus logarithmic concentration using SigmaPlot version 15.0. The IC₅₀ values are presented as the mean $\pm$ SD of three independent experiments. The selectivity index (SI) was calculated as the ratio of the IC₅₀ value against Vero cells to that against each cancer cell line. An SI value greater than 3 was considered indicative of selective cytotoxicity toward cancer cells.^{28,29}

Statistical analysis

Data are presented as the mean $\pm$ standard deviation (SD) of three independent experiments, with each concentration tested in six replicate wells. IC₅₀ values were derived from the corresponding concentration–response curves.

Results and Discussion

Structural characterization of the isolated compounds

The CH₂Cl₂ extract of the young fruits of *N. crenulata* exhibited selective cytotoxicity toward KB and MCF-7 cells, with SI values of 11.25 and 6.07, respectively. Purification of this extract yielded three known prenylated benzoyltyramine alkaloids: acidissimin (**1**), acidissimin epoxide (**2**), and acidissiminol (**3**) (Figure 1). Their structures were assigned from IR, NMR, and mass spectrometric data acquired in the present study; previously published values were used only for comparison and identification of the known compounds. Although compounds **1–3** have been reported from the fruits of the wood apple, *Limonia acidissima* (Rutaceae),^{26,27} this is, to the best of our knowledge, their first reported occurrence in *N. crenulata*.

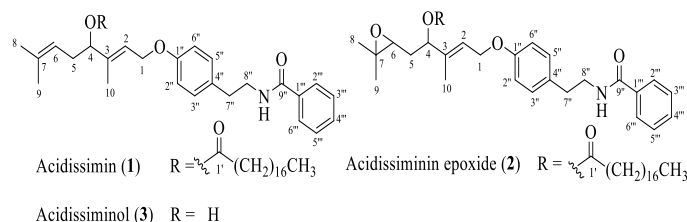


Figure 1: Structures of known prenylated benzoyltyramine alkaloids **1–3**

Compound **1** was isolated as a white solid, mp 64–66 °C. Its molecular formula was established as $C_{43}H_{65}NO_4$ from the HR-ESIMS ion at m/z 682.4782 $[M + Na]^+$ (calcd for $C_{43}H_{65}NNaO_4$, 682.4805), corresponding

to 12 degrees of unsaturation. The IR absorptions at 3348, 1731, and 1639 cm^{-1} indicated the presence of an NH group, an ester carbonyl, and an amide carbonyl, respectively. The ¹³C NMR, DEPT, and HSQC spectra accounted for all 43 carbon atoms, comprising four methyl, 20 methylene, 12 methine, and seven nonprotonated carbons. Two carbonyl resonances at δ_c 173.0 and 167.4 were assigned to an ester carbonyl (C-1') and an amide carbonyl (C-9''), respectively. The ¹H NMR spectrum exhibited a five-proton aromatic pattern at δ_H 7.69 (2H), 7.47 (1H), and 7.39 (2H), together with the corresponding carbon signals at δ_c 126.8, 128.5, 131.3, and 134.7, characteristic of a monosubstituted benzoyl group. In addition, two pairs of aromatic doublets at δ_H 6.84 and 7.13 (each 2H, $J = 8.6$ Hz) indicated a para-disubstituted phenyl ring. The methylene resonances at δ_H/δ_c 2.86/34.8 (H-7''/C-7'') and 3.67/41.3 (H-8''/C-8''), together with an NH signal at δ_H 6.18, supported an *N*-benzoyltyramine framework. This assignment was further substantiated by the H-7''/H-8'' COSY correlation and HMBC correlations connecting the ethylene unit to the para-disubstituted aromatic ring and the amide carbonyl. Long-range correlations from H-2'''/H-6''' to C-9'' established attachment of the benzoyl ring to the amide carbonyl. The remaining resonances defined an oxygenated geranyl-derived unit. The oxymethylene H-1 at δ_H 4.55 correlated in the HSQC spectrum with C-1 at δ_c 64.5, while the olefinic methines H-2 and H-6 appeared at δ_H/δ_c 5.71/122.7 and 5.00/118.9, respectively. An additional oxygenated methine was observed at δ_H/δ_c 5.15/77.5 (H-4/C-4). The COSY correlations of H-1/H-2 and H-4/H-5/H-6 defined the two proton sequences within this unit. The quaternary olefinic carbons at δ_c 137.7 (C-3) and 134.7 (C-7), together with the methyl resonances for H-8, H-9, and H-10, completed the 3,7-dimethylocta-2,6-dienyl framework. Its connection to the tyramine aromatic ring through an ether linkage was established by the HMBC correlation from H-1 to the oxygenated aromatic carbon C-1'' (δ_c 157.4). The *E* geometry of the C-2/C-3 double bond was supported by the relatively shielded resonance of the olefinic methyl carbon C-10 at δ_c 13.1, consistent with the reported data for related acidissiminol derivatives.^{26,27}

The ester substituent was identified as a stearyloxy group from the signals of an ester carbonyl at δ_c 173.0, an α -methylene at δ_H/δ_c 2.28/34.8 (H-2'/C-2'), a β -methylene at δ_H/δ_c 1.63/25.0 (H-3'/C-3'), an extended methylene envelope at δ_H 1.25, and a terminal methyl group at δ_H/δ_c 0.87/14.1 (H-18'/C-18'). The HMBC correlation from H-4 to C-1' located the stearyloxy moiety at C-4. Accordingly, the spectroscopic evidence established compound **1** as a C-4-stearylated benzoyltyramine bearing an oxygenated geranyl unit. Comparison of its physicochemical and NMR data with those reported in the literature confirmed the identity of compound **1** as acidissimin.²⁶

Compound **2** was isolated as a white solid, mp 105–107 °C. Its molecular formula, $C_{43}H_{65}NO_5$, was established from the HR-ESIMS ion at m/z 698.4733 $[M + Na]^+$ (calcd for $C_{43}H_{65}NNaO_5$, 698.4754), indicating the incorporation of one additional oxygen atom relative to **1**. The IR absorptions at 3345, 1729, and 1639 cm^{-1} supported the presence of NH, ester carbonyl, and amide carbonyl groups, respectively. The NMR data retained the benzoyltyramine, stearyl ester, and C-2/C-3 olefinic features of **1**, but lacked the C-6/C-7 alkene signals at δ_H/δ_c 5.00/118.9 and δ_c 134.7. These resonances were replaced by an oxirane methine at δ_H/δ_c 2.70/60.7 (H-6/C-6) and a nonprotonated oxirane carbon at δ_c 57.8 (C-7). The H-5/H-6 COSY correlation and HMBC cross-peaks from H-8/H-9 to C-6 and C-7 located the epoxide at C-6/C-7. The HMBC correlation from H-4 (δ_H 5.36) to the ester carbonyl C-1' (δ_c 172.8) confirmed retention of the C-4 stearyloxy group. Accordingly, **2** was identified as acidissimin epoxide by comparison with the reported spectroscopic data.²⁷

Compound **3** was obtained as a white solid, mp 86–88 °C. The molecular formula $C_{25}H_{31}NO_3$ was deduced from its HR-ESIMS ion at m/z 416.2197 $[M + Na]^+$ (calcd for $C_{25}H_{31}NNaO_3$, 416.2196), accounting for 11 degrees of unsaturation. Its NMR profile retained the benzoyltyramine and geranyl-derived features of acidissimin (**1**), but showed no resonances attributable to the stearyl chain or its ester carbonyl. Consistent with deacylation at C-4, H-4 shifted upfield from δ_H 5.15 in **1** to δ_H 4.06 in **3** and correlated by HSQC with the oxygenated methine C-4 at δ_c 76.2. The H-4/H-5/H-6 COSY sequence confirmed that this hydroxy-bearing methine remained embedded within the

monoterpenoid chain, whereas the remaining HSQC and HMBC correlations preserved the same aryl ether and benzoyltyramine connectivity established for **1**. The broad IR absorption at 3330 cm⁻¹ and the absence of an ester-carbonyl band further supported the presence of a free hydroxy group. Accordingly, compound **3** was assigned as the C-4 deacylated analogue of **1** and identified as acidissiminol by comparison with the reported spectroscopic data.²⁷

Cytotoxic activity evaluation of the extracts and isolated compounds

The cytotoxic activities of the extracts and compounds **1–3** were evaluated against KB and MCF-7 cancer cells, while Vero cells were included to assess selectivity toward non-cancerous cells (Table 1). Among the four extracts, the CH₂Cl₂ extract showed the strongest cytotoxicity against both KB and MCF-7 cells, with IC₅₀ values of 15.08 ± 0.51 and 27.92 ± 6.19 µg/mL, respectively. It also exhibited considerable selectivity, with corresponding SI values of 11.25 and 6.07. The hexane extract was less active, with IC₅₀ values of 30.54 ± 3.00 µg/mL against KB cells and 67.51 ± 5.78 µg/mL against MCF-7 cells, but remained selective toward both cell lines.

Table 1: Cytotoxic activities of young-fruit extracts of *Naringi crenulata* (Roxb.) Nicolson and the isolated compounds **1–3**.

Extract or compound	KB		MCF-7		Vero
	IC ₅₀ (µg/mL)	SI	IC ₅₀ (µg/mL)	SI	IC ₅₀ (µg/mL)
Hexane extract	30.54 ± 3.00	10.89	67.51 ± 5.78	4.92	332.67 ± 16.99
CH ₂ Cl ₂ extract	15.08 ± 0.51	11.25	27.92 ± 6.19	6.07	169.65 ± 26.62
Acetone extract	85.07 ± 4.30	9.54	245.83 ± 18.10	3.30	811.85 ± 1.65
MeOH extract	858.04 ± 9.14	>1.17	>1000	ND	>1000
Compound 1	69.11 ± 1.41	>1.45	>100	ND	>100
Compound 2	6.11 ± 0.82	>16.37	>100	ND	>100
Compound 3	0.79 ± 0.06	6.10	0.87 ± 0.07	5.54	4.82 ± 0.66
Doxorubicin	0.078 ± 0.003	11.79	0.19 ± 0.01	4.84	0.92 ± 0.12

Note: Values are expressed as the mean ± SD of three independent experiments. Extracts and isolated compounds were tested at concentrations of up to 1,000 and 100 µg/mL, respectively. IC₅₀, concentration required to reduce cell viability by 50%; SI, selectivity index, calculated as IC₅₀ (Vero)/IC₅₀ (cancer cell); KB, human oral epidermoid carcinoma cells; MCF-7, human breast adenocarcinoma cells; Vero, African green monkey kidney cells; SD, standard deviation; ND, not determined. IC₅₀ values preceded by ">" indicate that 50% inhibition was not achieved at the highest concentration tested. SI values preceded by ">" represent lower-bound estimates derived from the corresponding IC₅₀ limit for Vero cells. Doxorubicin was used as the positive control.

Although the acetone extract displayed weaker cytotoxicity, it showed SI values of 9.54 and 3.30 against KB and MCF-7 cells, respectively. In contrast, the MeOH extract exhibited only weak cytotoxicity against KB cells, while its IC₅₀ values against MCF-7 and Vero cells could not be determined within the concentration range tested.

The present findings add to previous evidence of the cytotoxic potential of structurally related prenylated benzoyltyramine alkaloids. Atalantums A–G from *A. monophylla* showed cytotoxicity against cholangiocarcinoma cell lines,¹⁸ while atalantums H–K and related benzoyltyramines were evaluated against HeLa, HCT116, and MCF-7 cells, with Vero cells included for selectivity assessment.¹⁹ Acidissiminol epoxide from *A. monophylla* was also reported to induce apoptosis and G0/G1 cell-cycle arrest in MCF-7 cells.²⁰ Direct comparisons of potency should nevertheless be made cautiously because the compounds, cell lines, exposure periods, and assay conditions differed among studies.

In the present study, compound **3** was the most potent isolated compound, with IC₅₀ values of 0.79 ± 0.06 and 0.87 ± 0.07 µg/mL against KB and MCF-7 cells, respectively. Its corresponding SI values of 6.10 and 5.54 indicated relative selectivity toward both cancer cell lines under the conditions of this in vitro assay, although its IC₅₀ value against Vero cells (4.82 ± 0.66 µg/mL) also demonstrated appreciable cytotoxicity toward non-cancerous cells. Compound **2** showed lower potency but greater selectivity toward KB cells (IC₅₀ = 6.11 ± 0.82 µg/mL; SI > 16.37), whereas its IC₅₀ values against MCF-7 and Vero cells were greater than 100 µg/mL. Compound **1** was less active against KB cells (IC₅₀ = 69.11 ± 1.41 µg/mL; SI > 1.45) and likewise exhibited IC₅₀ values greater than 100 µg/mL against MCF-7 and Vero cells. The markedly greater activity of acidissiminol (**3**) than that of its C-4-stearoylated analogue acidissimin (**1**) provides a preliminary indication that the free C-4 hydroxy group and/or the absence of the long-chain acyl substituent may contribute to cytotoxic activity; however, additional analogues and mechanistic studies are required to establish this preliminary structure–activity relationship. Thus, the present findings extend the known cytotoxic profile of benzoyltyramine alkaloids and provide the first evidence for the cytotoxicity and selectivity of compounds **1–3** isolated from *N. crenulata* against KB and MCF-7 cells. Vero cells are derived from African green monkey kidney and are not human non-malignant counterparts of the tissues represented by KB or MCF-7 cells. Accordingly, the SI values indicate relative selectivity only within the tested cell models and should not be interpreted as evidence of therapeutic safety.

Conclusion

Phytochemical investigation of the young fruits of *N. crenulata* led to the isolation of three known prenylated benzoyltyramine alkaloids: acidissimin (**1**), acidissimin epoxide (**2**), and acidissiminol (**3**). Although previously reported from *L. acidissima*, these compounds are reported here for the first time from *N. crenulata*. Among the extracts, the CH₂Cl₂ extract showed the strongest and most selective cytotoxicity toward KB and MCF-7 cells. Of the isolated compounds, acidissiminol (**3**) was the most potent against both cell lines, with IC₅₀ values of 0.79 ± 0.06 and 0.87 ± 0.07 µg/mL and SI values of 6.10 and 5.54, respectively. Acidissimin epoxide (**2**) was less potent than **3** but showed greater selectivity toward KB cells (IC₅₀ = 6.11 ± 0.82 µg/mL; SI > 16.37). This study expands the known distribution of prenylated benzoyltyramine alkaloids within the Rutaceae and provides the first evaluation of the cytotoxicity and selectivity of compounds **1–3** against KB, MCF-7, and Vero cells. These findings represent preliminary in vitro cytotoxicity data. Because compound **3** also showed appreciable cytotoxicity toward Vero cells, further evaluation using appropriate human non-malignant cell models and mechanistic studies is required before its anticancer potential or safety can be assessed.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

This research was partially funded by Phetchaburi Rajabhat University through the Fundamental Fund for the 2026 fiscal year, supported by the National Science Research and Innovation Fund (NSRF) (Grant No. 222487). The authors gratefully acknowledge the Department of Chemistry, Faculty of Science, Silpakorn University, for the NMR measurements and the Department of Chemistry, Faculty of Science, Mahidol University, for the mass spectrometric analyses.

References

1. Lim MW, Aroua MK, Gew LT. Thanaka (*H. crenulata*, *N. crenulata*, *L. acidissima* L.): A systematic review of its chemical, biological properties and cosmeceutical

- applications. *Cosmetics*. 2021; 8(3): 68. <https://doi.org/10.3390/cosmetics8030068>
2. Saensouk P, Saensouk S, Boonma T, Hanchana K, Rakarcha S, Maknoi C, Chanthavongsa K, Jitpromma T. Ecological analysis and ethnobotanical evaluation of plants in Khanthararat Public Benefit Forest, Kantarawichai District, Thailand. *Forests*. 2025; 16(6): 1012. <https://doi.org/10.3390/f16061012>
 3. Venkatachalam P, Thilagavathi C, Ramesh T, Lenin M. Preliminary phytochemical evaluation of methanol extract leaves of *Naringi crenulata* (Wild). *Bull Environ Pharmacol Life Sci*. 2021; 10(10): 220-224.
 4. Sekhar J, Penchala PG, Sudarsanam G, Prasad GP. Ethnic information on treatments for snakebites in Kadapa District of Andhra Pradesh. *Life Sci Leafl*. 2011; 12: 368-375.
 5. Babu K, Srinivasaprabhu DK. Pharmacognostic and phytochemical evaluation of leaf and stem of *Naringi crenulata* (Roxb.) Nicolson. *J Pharmacogn Phytochem*. 2024; 13(5): 254-259. <https://doi.org/10.22271/phyto.2024.v13.i5c.15081>
 6. Seiverling EV, Trubiano JP, Williams JC, Ahrens HT, Craft DW, England MR. Analysis of the antimicrobial properties of Thanaka, a Burmese powder used to treat acne. *J Biosci Med*. 2017; 5(7): 1-6. <https://doi.org/10.4236/jbm.2017.57001>
 7. Di Nicolantonio L, Trebbi G, Vargas Peregrina D, Rashad M, Censi R, Zara S, Di Martino P, Gigliobianco MR. Development and characterization of a new cosmetic formulation enriched with optimized Thanaka bark extract (*H. crenulata*). *J Drug Deliv Sci Technol*. 2025; 113: 107343. <https://doi.org/10.1016/j.jddst.2025.107343>
 8. Aung HM, Kanpipit N, Thapphasaraphong S. Effects of Thanaka (*Hesperethusa crenulata*) stem bark extract on collagen activation and anti-melanogenesis for cosmetic applications. *Trop J Nat Prod Res*. 2024; 8(1): 5852-5860. <https://doi.org/10.26538/tjnpr/v8i1.21>
 9. Ramachandran VS, Rajith NP. Ethnomedicines of Kurichyas, Kannur district, Western Ghats, Kerala. *Indian J Nat Prod Resour*. 2010; 1(2): 249-253.
 10. Padal SB, Murty PP, Rao DS, Venkaiah M. Ethnomedicinal plants from Paderu division of Visakhapatnam District, A.P., India. *J Phytol*. 2010; 2(8): 70-91.
 11. Senthilkumar M, Gurumoorthi P, Janardhanan K. Some medicinal plants used by Irular, the tribal people of Marudhamalai hills, Coimbatore, Tamil Nadu. *Nat Prod Radiance*. 2006; 5(5): 382-388.
 12. Niu XM, Li SH, Peng LY, Lin ZW, Rao GX, Sun HD. Constituents from *Limonia crenulata*. *J Asian Nat Prod Res*. 2001; 3(4): 299-311. <https://doi.org/10.1080/10286020108040370>
 13. Singha S, Kruakaew S, Yahuafai J, Thongnest S, Sutthivaiyakit S. Limonoids and other constituents of the stem of *Naringi crenulata* (Roxb.) Nicholson. *Nat Prod Res*. 2025; 1-11. <https://doi.org/10.1080/14786419.2025.2566473>
 14. Nayar MNS, Bhan MK. Coumarins and other constituents of *Hesperethusa crenulata*. *Phytochemistry*. 1972; 11(11): 3331-3333. [https://doi.org/10.1016/S0031-9422\(00\)86402-X](https://doi.org/10.1016/S0031-9422(00)86402-X)
 15. Nayar MNS, Sutar CV, Bhan MK. Alkaloids of the stem bark of *Hesperethusa crenulata*. *Phytochemistry*. 1971; 10(11): 2843-2844. [https://doi.org/10.1016/S0031-9422\(00\)97306-0](https://doi.org/10.1016/S0031-9422(00)97306-0)
 16. Chatterjee A, Sarkar S, Shoolery JN. 7-Phenylacetoxy coumarin from *Limonia crenulata*. *Phytochemistry*. 1980; 19(10): 2219-2220. [https://doi.org/10.1016/S0031-9422\(00\)82233-5](https://doi.org/10.1016/S0031-9422(00)82233-5)
 17. Yesudanam S, Jasmin TS, Jatt RK. GC-MS analysis of bioactive compounds using methanolic extracts of *Naringi crenulata* (Roxb.) Nicolson leaves. *Indo Am J Pharm Sci*. 2021; 8(9): 224-232. <https://doi.org/10.5281/zenodo.5515265>
 18. Sribuhom T, Boueroy P, Hahnvajanawong C, Phatchana R, Yenjai C. Benzoyltyramine alkaloids atalantums A-G from the peels of *Atalantia monophylla* and their cytotoxicity against cholangiocarcinoma cell lines. *J Nat Prod*. 2017; 80(2): 403-408. <https://doi.org/10.1021/acs.jnatprod.6b00908>
 19. Sombatsri A, Thummanant Y, Sribuhom T, Wongphakham P, Senawong T, Yenjai C. Atalantums H-K from the peels of *Atalantia monophylla* and their cytotoxicity. *Nat Prod Res*. 2020; 34(15): 2124-2130. <https://doi.org/10.1080/14786419.2019.1576042>
 20. Sudta P, Yahuafai J, Prapasanol V, Wangchuk P. Cytotoxic and apoptotic effects of compounds isolated from *Atalantia monophylla* peels on human cancer cell lines. *Asian Pac J Cancer Prev*. 2026; 27(3): 867-875. <https://doi.org/10.31557/APJCP.2026.27.3.867>
 21. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024; 74(3): 229-263. <https://doi.org/10.3322/caac.21834>
 22. Juthani R, Punatar S, Mittra I. New light on chemotherapy toxicity and its prevention. *BJC Rep*. 2024; 2: 41. <https://doi.org/10.1038/s44276-024-00064-8>
 23. Khan SU, Fatima K, Aisha S, Malik F. Unveiling the mechanisms and challenges of cancer drug resistance. *Cell Commun Signal*. 2024; 22(1): 109. <https://doi.org/10.1186/s12964-023-01302-1>
 24. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT; International Natural Product Sciences Taskforce. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov*. 2021; 20(3): 200-216. <https://doi.org/10.1038/s41573-020-00114-z>
 25. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod*. 2020; 83(3): 770-803. <https://doi.org/10.1021/acs.jnatprod.9b01285>
 26. Ghosh P, Bandyopadhyay AK, Thakur S, Akihisa T, Shimizu N, Tamura T, Matsumoto T. Acidissimin, a new tyramine derivative from the fruit of *Limonia acidissima*. *J Nat Prod*. 1989; 52(6): 1323-1326. <https://doi.org/10.1021/np50066a024>
 27. Ghosh P, Sil P, Das S, Thakur S, Kokke WCMC, Akihisa T, Shimizu N, Tamura T, Matsumoto T. Tyramine derivatives from the fruit of *Limonia acidissima*. *J Nat Prod*. 1991; 54(5): 1389-1393. <https://doi.org/10.1021/np50077a024>
 28. Chothiphirat A, Nittayaboon K, Kanokwiroon K, Srisawat T, Navakanitworakul R. Anticancer potential of fruit extracts from *Vatica diospyroides* Symington type SS and their effect on program cell death of cervical cancer cell lines. *ScientificWorldJournal*. 2019; 2019: 5491904. <https://doi.org/10.1155/2019/5491904>
 29. Yahuafai J, Onsrirawat P, Piyaviriyakul S, Nontakham J, Suthamnatpong N. Anticancer effect of Triphala extract on the hepatocellular carcinoma cells in mice. *J Pharm Pharmacogn Res*. 2023; 11(3): 448-454. https://doi.org/10.56499/jppres23.1602_11.3.448