

Isolation and Structural Elucidation of Coumarin and Flavonoids from *Citrus grandis* Linn. (Tubtim Siam Pomelo) and Their Biological Activities

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Abstract

Natural products from plants have the potential to exert antioxidant, anti-tyrosinase, antibacterial, and cytotoxic activities and have been widely used as ingredients in cosmetics, medicines, or supplementary food. Several types of secondary metabolites from the *Citrus* genus have been reported to exhibit interesting biological activity. Due to this lack of studies and biological activity of compounds from related species, it was considered important to investigate *Citrus grandis* Linn. (Tubtim Siam Pomelo) to be studied. The objective of this research was to isolate and elucidate the structures of chemical constituents and to evaluate their biological activity from the peels of *C. grandis* Linn. A coumarin (1) and 3 flavonoids (2 - 4) were isolated from the peels of *C. grandis* Linn. The structures of these compounds were elucidated by analyses of spectroscopic data as well as comparison of their spectroscopic data with the previously published data. Compounds 1 - 4 have been evaluated for antioxidant, anti-tyrosinase, antibacterial and cytotoxic activities. Compound 2 showed the highest inhibition in DPPH and tyrosinase with IC₅₀ values of 0.219 ± 0.011 and 0.033 ± 0.002 mg/mL, respectively. Interestingly, compound 1 - 4 were the first time being isolated from Tubtim Siam Pomelo.

Keywords: *Citrus grandis* Linn, Coumarins, Flavonoids, Antioxidant activity, Anti-tyrosinase activity, Antibacterial activity, Cytotoxic activity

Introduction

Bioactive compounds are widely used to treat diseases such as cancer, infectious diseases and various other dangerous diseases. Bioactive compounds can be found in plants, animals and microorganisms. Biochemical substances isolated from plants such as phenolic, flavonoids, anthocyanins, and terpenoids have high potential for antioxidant, antimicrobial, anti-inflammatory, anti-tyrosinase and anticancer effects. *Citrus* plants are also enriched with various active compounds that are very beneficial to health such as Vitamin C, carotenoids, minerals, essential oils, flavonoids, acridone alkaloids, limonoids, coumarins and triterpenoids [1-4]. In Asian countries, citrus fruits, such as lime, lemon and pomelo are widely available and regularly consumed as whole fruits, fruit juices and preserved snacks. It has been used to treat many symptoms of disease in humans such as coughs, skin inflammation, muscle pains, stomach upsets, and ringworm infections [5]. Additionally, citrus plants have proven antimicrobial [6,7], antioxidant [8-10], anti-inflammatory [11], anti-tyrosinase [12,13], antihyperlipidemic [14,15] and anticancer properties [16,17].

Citrus grandis Linn. or *C. maxima* Merr., commonly known as Pomelo, belongs to the family Rutaceae. It is widely cultivated in many countries because of its good taste and nutritional benefits. In

traditional medicine, various parts of this plant are practiced in folk medicine such as skin disorders, headache, stomach pain, cough, swelling, epilepsy, asthma, leprosy and hiccup [18,19]. Limonoids, flavanones, polyphenol contents and antioxidant activity were present in 7 pummelo cultivars, including Kao Numpueng, Thong Dee, Kao Pean, Kao Yai, Tha Khoi, Kao Tangkya and Pattavee. Two limonoids were identified as limonin and nomilin [20]. Tubtim Siam Pomelo (*C. grandis* Linn) is grown in Pak Phanang, Nakhon Si Thammarat, Thailand. It is a tree with edible fruits. The peels of *C. grandis* Linn. are considered part of the waste products that are generally discarded. Interestingly, the chemical constituents and their biological activity from the peels of *C. grandis* Linn. (Tubtim Siam Pomelo) have not been reported.

Therefore, the objectives of this study were to assess the isolation, structure elucidation and biological activity from the peels of Tubtim Siam Pomelo. Four compounds (1 - 4) which are reported in this study. The antioxidant, anti-tyrosinase, antimicrobial and cytotoxic activities of all the pure compounds (1 - 4) were analyzed.

Materials and methods

General experimental procedures

Melting points were determined on a digital Electrothermal Melting Point Apparatus (Stone, Staffordshire, ST15 0SA, UK). Optical rotations were determined on a JASCO P-1020 polarimeter (JASCO, Tokyo, Japan). A PerkinElmer FT-IR FTS 165 spectrophotometer was utilized for measuring the IR spectra (Perkin-Elmer, Shelton, USA). The ^1H and ^{13}C NMR spectra were recorded on a FT-NMR Bruker Ultra ShieldTM 500 MHz and a Unity Inova Varian 500 MHz spectrometer using tetramethylsilane (TMS) as the internal standard. Quick column chromatography (QCC) was carried out on silica gel 60 GF254 (Merck). Column chromatography (CC) was performed by using silica gel 100 (70 - 230 Mesh ASTM, Merck). Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel 60 F254 (Merck). Solvents for extraction and chromatography (hexane, CH_2Cl_2 , acetone, EtOAc and MeOH) were distilled at their boiling ranges prior to use.

Extraction and isolation

The *C. grandis* (Tubtim Siam Pomelo) were collected from Amphoe Pak Phanang, Nakhon Si Thammarat, Thailand, in October 2021. The dried peels of *C. grandis* Linn. (Tubtim Siam Pomelo) (2.30 kg) was immersed in acetone at room temperature for 7 days. After evaporation, the acetone extract as a dark brown gum (40.40 g) was subjected to QCC over silica gel and eluted with a gradient solvent system of hexane, hexane/acetone, acetone, acetone/MeOH and finally with pure methanol to furnish 13 fractions (A - M). Fraction D (2.8970 g) was further fractionated by CC with hexane:acetone (4:1, v/v) that yielded 9 subfractions (D1 - D9). Subfraction D5 (232.5 mg) was further separated by CC with hexane: CH_2Cl_2 :acetone (7:2:1, v/v) to afford 6 fractions (D5A-D5F). Subfraction D5D (28.9 mg) was further purified by CC hexane: CH_2Cl_2 :acetone (7:2:1, v/v) to afford a yellow viscous solid 1 (7.8 mg). Fraction G (3.5605 g) was further fractionated by CC with hexane: CH_2Cl_2 :acetone (7:2:1, v/v) that yielded 12 subfractions (G1 - G12). Subfraction G3 (390.6 mg) was further separated by CC with hexane:acetone (7:3, v/v) to afford 9 fractions (G3A - G3I). Subfraction G3D (88.9 mg) was further purified by CC hexane: CH_2Cl_2 :EtOAc (7:2:1, v/v) to afford 7 fractions (G3D1 - G3D7). Subfraction G3D4 (31.9 mg) was further purified by CC hexane: CH_2Cl_2 :EtOAc (7:2:1, v/v) to give a yellow solid 2 (6.9 mg). Fraction H (3.7142 g) was further fractionated by CC with hexane:acetone (3:2, v/v) that yielded 9 subfractions (H1 - H9). Subfraction H5 (387.8 mg) was further separated by CC with hexane:acetone (7:3, v/v) to give 12 subfractions (H5A - H5L). Subfraction H5C (45.6 mg) was further separated by CC with hexane: CH_2Cl_2 :EtOAc (3:1:1, v/v) to give a yellow solid 3 (6.5 mg). Fraction L (2.8902 g) was subjected to a reverse phase column with H_2O :MeOH (1:1, v/v) that yielded 6 subfractions (L1 - L4). Subfraction L2 (1.6223 g) was subjected to a reverse phase column with H_2O :MeOH (1:1, v/v) that to give a yellow solid 4 (170.9 mg).

8-(2,3-dihydroxy-3-methylbutyl)-7-methoxy-2H-1-benzopyran-2-one (1)

^1H NMR (CDCl_3 , 500 MHz) δ : 1.31 (3H, s, H-4'), 1.32 (3H, s, H-5'), 3.01 (1H, dd, 14.0, 10.0, H-1 β '), 3.07 (1H, dd, 14.0, 2.5, H-1 α '), 3.63 (1H, dd, 10.0, 2.5, H-2'), 3.93 (3H, s, 7-OMe), 6.22 (1H, d, 9.5, H-3), 6.87 (1H, d, 8.5, H-6), 7.34 (1H, d, 8.5, H-5), 7.64 (1H, d, 9.5, H-4). ^{13}C NMR (CDCl_3 , 125 MHz) δ : 24.2 (C-4'), 25.6 (C-1'), 26.0 (C-5'), 56.2 (7-OMe), 73.2 (C-3'), 78.3 (C-2'), 107.5 (C-6), 112.8 (C-3), 113.1 (C-10), 115.8 (C-8), 127.0 (C-5), 144.2 (C-4), 153.3 (C-9), 160.6 (C-7), 161.6 (C-2).

5-hydroxy-6,7,8,3',4'-pentamethoxyflavone (2)

¹H NMR (acetone-*d*₆, 500 MHz) δ : 3.88 (3H, *s*, 6-OMe), 3.93 (3H, *s*, 4'-OMe), 3.97 (3H, *s*, 3'-OMe), 3.99 (3H, *s*, 8-OMe), 4.08 (3H, *s*, 7-OMe), 6.78 (1H, *s*, H-3), 7.16 (1H, *d*, 10.0, H-5'), 7.62 (1H, *d*, 2.5, H-2'), 7.72 (1H, *dd*, 10.0, 2.5, H-6'), 12.76 (1H, *s*, 5-OH). ¹³C NMR (acetone, 125 MHz) δ : 56.3 (3'-OMe), 56.3 (4'-OMe), 61.0 (6-OMe), 61.9 (7-OMe), 62.4 (8-OMe), 104.3 (C-3), 107.6 (C-4a), 110.1 (C-2'), 112.6 (C-5'), 121.0 (C-6'), 124.3 (C-1'), 134.0 (C-8), 137.4 (C-6), 146.6 (C-8a), 150.3 (C-5), 150.6 (C-3'), 153.9 (C-4'), 154.0 (C-7), 165.0 (C-2), 183.9 (C-4).

5,6,7,8,4'-pentamethoxyflavone (3)

¹H NMR (CDCl₃, 500 MHz) δ : 3.88 (*s*, 5'-OMe), 3.95 (6-OMe/3'-OMe), 4.02 (7-OMe), 4.11 (8-OMe), 6.61 (1H, *s*, H-3), 7.01 (1H, *d*, 9.0, H-3'/H-5'), 7.88 (1H, *d*, 9.0, H-2'/H-6'). ¹³C NMR (CDCl₃, 125 MHz) δ : 55.4, 61.8, 61.9, 62.2, 62.4 (5xOMe), 106.2 (C-3), 114.7 (C-3'/C-5'), 123.6 (C-1'), 128.1 (C-2'/C-6'), 138.2 (C-6), 144.4 (C-8), 147.9 (C-8a), 148.4 (C-5), 151.8 (C-7), 162.7 (C-2), 177.6 (C-4), 114.7 (C-4a).

Naringin (4)

¹H NMR (acetone -*d*₆, 500 MHz) δ : 1.14 (3H, *d*, 6.6, H-6'''), 2.78 (1H, *dd*, 17.3, 3.0, H-3b), 3.26 (1H, *dd*, 17.3, 12.8, H-3a), 3.41-3.98 (*m*, rhamnosyl and glucosyl protons), 5.18 (1H, *d*, 7.5, H-1''), 5.34 (1H, *brm*, H-1'''), 5.51 (1H, *dd*, 12.8, 3.0, H-2), 6.16 (1H, *d*, 2.5, H-8), 6.17 (1H, *d*, 2.5, H-6), 6.90 (1H, *d*, 8.5, H-3'/H-5'), 7.40 (1H, *d*, 8.5, H-2'/H-6'), 12.03 (1H, *s*, 5-OH). ¹³C NMR (acetone-*d*₆, 125 MHz) δ : 17.5 (C-6'''), 197.2 (C-4), 103.7 (C-4a), 163.8 (C-5), 95.4 (C-6), 165.4 (C-7), 95.3 (C-8), 163.3 (C-8a), 129.5 (C-1'), 128.3 (C-2'), 115.4 (C-3'), 158.0 (C-4'), 115.4 (C-5'), 128.3 (C-6'), 98.2 (C-1''), 76.8 (C-2''), 70.3* (C-3''), 70.9* (C-4''), 76.7 (C-5''), 61.4 (C-6''), 100.7 (C-1'''), 76.7* (C-2'''), 71.4 (C-3'''), 72.8 (C-4'''), 68.4 (C-5'''), 17.5 (C-6''') *exchangeable position.

Evaluation of antioxidant activity of pure compounds

Stock solution (0.076 mM) of DPPH was prepared by dissolving 0.003 g crystalline powder in 100 mL of 95 % methanol. A standard solution of Trolox was also prepared by dissolving 1 mg of crystalline Trolox in 1 mL 95 % methanol. The DPPH assay followed the modified method [21]. Radical scavenging activities were read at an absorbance of 517 nm and the percentage inhibition was calculated using the formula: ((OD values of control - OD values of sample/OD values of control well)×100). The experiment was conducted in triplicate for 2 independent trials.

Evaluation of anti-tyrosinase activity of pure compounds

The assay was carried out in a 96-well microtiter plate. In each well, 40 μ L of pure compounds, 80 μ L of phosphate buffer (0.1 M, pH 6.8), 40 μ L of tyrosinase enzyme (60 units/mL), and 40 μ L of L-DOPA (3.5 mM) were added. The mixture was incubated at 37 °C for 10 min and the absorbance was measured at 475 nm. The percentage of tyrosinase inhibition was calculated as follows:

$$\text{Tyrosinase inhibition (\%)} = (\text{Absorbance of test} / \text{Absorbance of control}) \times 100$$

Evaluation of antibacterial activity of pure compounds

Clinical isolates of methicillin-resistant *Staphylococcus aureus* NPRCoE 160801 were obtained from Songklanagarind Hospital (Ethical Approval No. REC 59-241-19-6). Reference strains including Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), Gram-negative bacteria (*Escherichia coli* ATCC 25922) were used in the study. Bacterial strains were subculture overnight at 37 °C on Mueller-Hinton agar and Sabouraud dextrose agar, respectively. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of gold nanoparticles were determined by following Clinical and Laboratory Standards Institute guidelines [22]. Two-fold broth microdilution method of the nanoparticles and antibiotics in Mueller Hinton broth for bacteria and RPMI 1640 medium for fungi was prepared in a sterile 96-well plate to obtain the final concentrations ranging from 5 - 80 μ g/mL. The microbial suspensions were adjusted to a 0.5 McFarland standard at OD 600 nm in normal saline solution. The tested pathogens were added to the 96-well plate and incubated at 37 °C for 18 h. MIC values were determined as the lowest gold nanoparticles concentration inhibiting microbial growth. Minimum bactericidal concentration (MBC) were defined as the lowest concentration of the gold nanoparticles that showed no colony growth on the culture medium.

Cell culture conditions and *in vitro* cytotoxicity testing

The breast cancer cell lines (MCF-7) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Colorectal carcinoma (HT-29) cell lines were provided by Dr. Surasak Sangkhathat (Faculty of Medicine, PSU, Thailand). MCF-7 cells were grown in RPMI 1640 (Invitrogen). HT-29 cell was grown in DMEM (Invitrogen). Each medium contained 10 % fetal bovine serum (Invitrogen) supplemented with 50 units/mL of penicillin (Invitrogen) and 50 $\mu\text{g/mL}$ of streptomycin (Invitrogen). All cells were incubated at 37 °C in an atmosphere of 5 % CO_2 and 95 % relative humidity. The cytotoxicity of the studied extracts and fractions were measured by MTT assay as previously described. An IC_{50} value less than 20 $\mu\text{g/mL}$ was considered to have *in vitro* cytotoxic activity [23]. Doxorubicin, a chemotherapeutic drug, was used as a positive control. The selectivity index (SI) was used to determine the selectivity of the extract as previously described [24]. The SI calculated the IC_{50} value of the extract on normal cells (L929) divided by the IC_{50} value of the extract on each cancer cells. SI value higher than 3 was considered as a prospective anticancer.

Results and discussion

Spectroscopic analysis

Extraction and purification of the acetone extract from the peels of *Citrus grandis* Linn. resulted in the isolation of 4 compounds (1 - 4) as shown in **Figure 1**. The structures of these compounds were elucidated by analyses of spectroscopic data analysis.

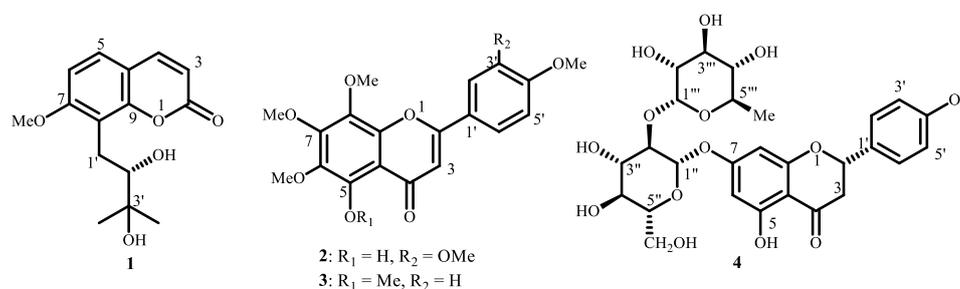


Figure 1 The chemical constituents from the peels of *C. grandis* Linn.

Compound 1 was obtained as a yellow viscous ($[\alpha]_{\text{D}}^{28} -34.7^\circ$ (c 0.34, CHCl_3). The IR spectrum showed the absorption band of $\text{C}=\text{O}$ stretching at $1,704.20\text{ cm}^{-1}$. The ^{13}C NMR spectrum (**Figure 2**) showed the resonances of a carbonyl ester carbon: δ 161.6 (C-2), 4 quaternary aromatic carbons: δ 160.6 (C-7), 115.8 (C-8), 153.3 (C-9), 113.1 (C-10), one oxy-quaternary aromatic carbon: δ 73.2 (C-3'), 5 methine carbons: δ 112.8 (C-3), 144.2 (C-4), 127.0 (C-5), 107.5 (C-6), 78.3 (C-2'), one methylene carbon: δ 25.6 (C-1'), one methoxy carbons: δ 56.2 (7-OMe), and 2 methyl carbons: δ 24.2 (C-4'), 26.0 (C-5'). The ^1H NMR spectrum (**Figure 2**) showed characteristic signals of α, β -olefinic coumarin at δ 6.22 (d, 9.5, H-3) and δ 7.64 (d, 9.5, H-4). The resonances at δ 7.34 (d, 8.5) and δ 6.87 (d, 8.5) were identified for aromatic protons H-5 and H-6, respectively which were coupled with ortho coupling. The HMBC correlations of H-4 to C-5 (δ 127.0) and H-5 to C-4 (δ 144.2) confirmed that the olefinic proton H-4 was peri to the aromatic proton H-5 as shown in **Figure 3**. The presence of prenyl group was deduced from signals of nonequivalent methylene protons H-1' at δ 3.07 (dd, 14.0, 2.5) and δ 3.01 (dd, 14.0, 10.0), methine protons H-2' at 3.63 (dd, 10.0, 2.5) and 2 methyl protons H-4'/H-5' at δ 1.31 and δ 1.32. Two oxy-carbons C-2' and C-3' of the prenyl group were showed at δ 78.3 and δ 73.2, respectively. The methoxy proton resonated at δ 3.93 that was located at C-7 according to the correlation of H-5 and methoxy protons (7-OMe) showed correlations to C-7 (δ 160.6). In addition, the prenyl group was placed at C-8 and ortho with 7-OMe according to the HMBC correlations of H-1' to C-7 (δ 160.6). Therefore, compound 1 then was identified as 8-(2,3-dihydroxy-3-methylbutyl)-7-methoxy-2H-1-benzopyran-2-one]. Its spectroscopic data agreed with the previously reported data of (-) meranzin hydrate [25].

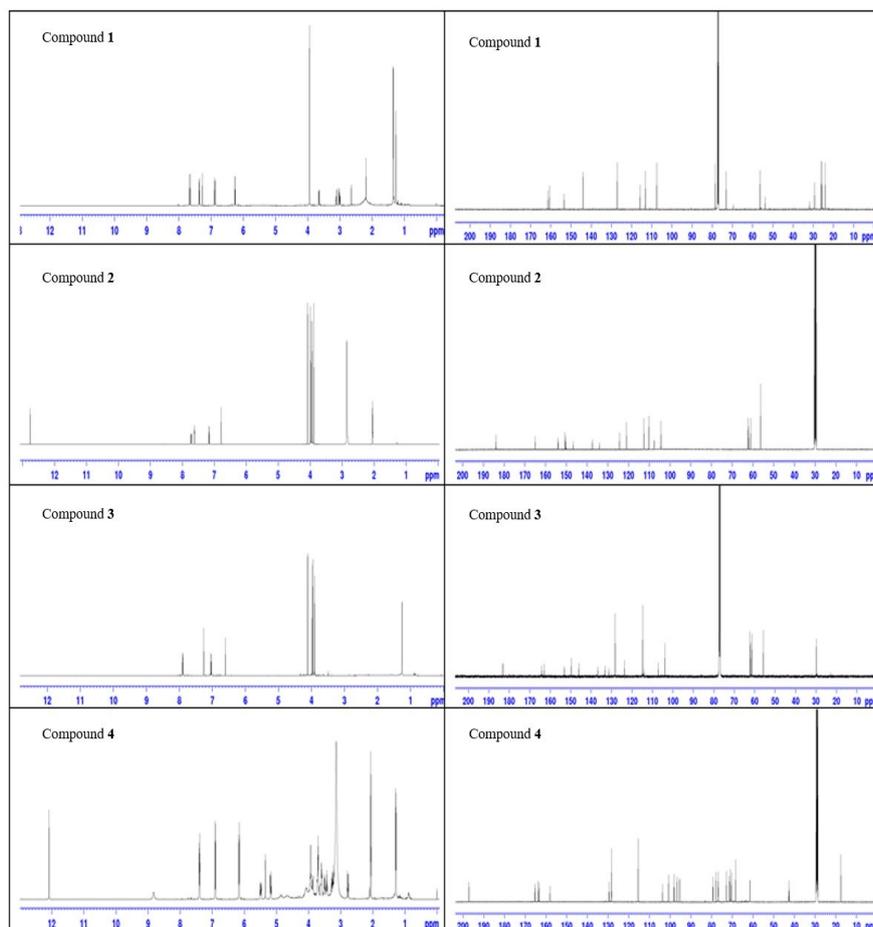


Figure 2 ^1H NMR and ^{13}C NMR spectra of the compounds (1 - 4).

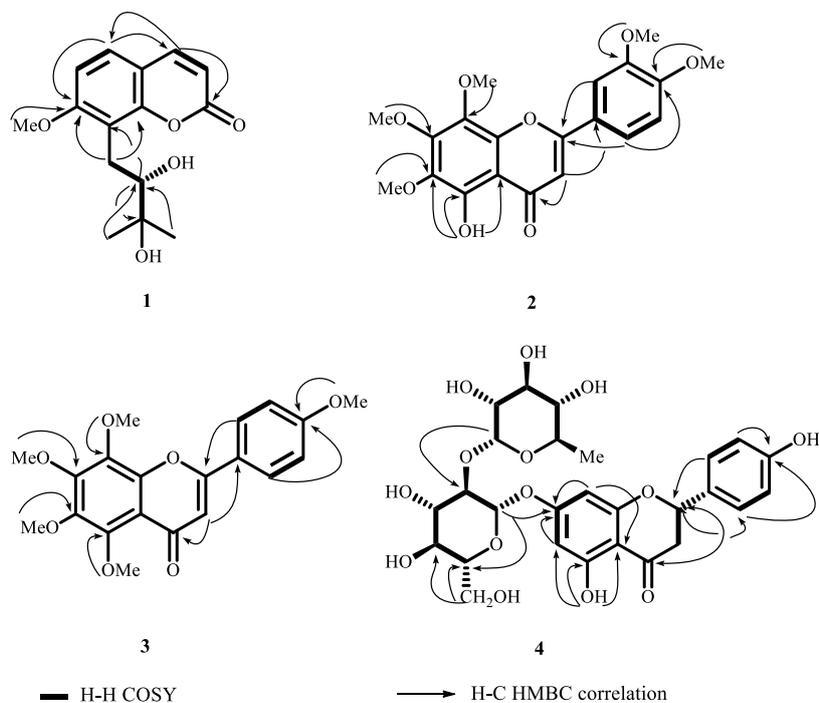


Figure 3 H-H COSY and Major HMBC correlation of the compounds (1 - 4).

Compounds 2 and 3 were indicated as pentamethoxy-substituted flavones. Compound 2 was obtained as a yellow solid, m.p. 142 - 143 °C. Its IR spectrum showed the stretching of hydroxyl (3,398.27 cm⁻¹) and chelated carbonyl groups (1,637.29 cm⁻¹). Its ¹H NMR spectrum (**Figure 2**) showed signals of an olefinic proton H-3 (δ 6.78, *s*), a chelated hydroxyl group 5-OH (δ 12.76), 5 methoxyl groups (δ 3.88, 3.93, 3.97, 3.99, 4.08), and ABX signals of aromatic protons H-5' (δ 7.16, *d*, 10.0), H-2' (7.62, *d*, 2.5) and H-6' (7.72, *dd*, 10.0, 2.5). Comprehensive analysis of 1D 2D NMR data, including the results of ¹H, ¹³C, COSY, HSQC, and HMBC experiments have been used to elucidate the structure of 2. Additionally, comparison NMR data with the previously reported. Compound 2 was 5-hydroxy-6,7,8,3',4'-pentamethoxyflavone which was known as 5-demethoxynobiletin [26].

Compound 3 was obtained as a yellow solid, m.p. 152 - 153 °C. Its IR spectrum showed the stretching of a carbonyl groups (1,651.50 cm⁻¹). Its ¹H-NMR spectrum (**Figure 2**) showed signals of an olefinic proton of flavone (δ 6.61, H-3), 5 methoxyl groups (δ 4.11, δ 4.02, δ 3.88 and δ 3.95 \times 2), a *para*-substituted B ring (H-2'/H-6', δ 7.88, *d*, *J* = 9.0 Hz; H-3'/H-5', δ 7.01, *d*, *J* = 9.0 Hz). The structures of compound 3 was confirmed by 1D and 2D NMR. The comparison these data those the previously reported, it was clear that the 3 is 5,6,7,8,4'-pentamethoxy-flavone which was known as tangeretin [26].

Compound 4 was obtained as a yellow solid, m.p. 168–169 °C. Its IR spectrum showed the stretching of hydroxyl (3,305.90 cm⁻¹) and chelated carbonyl groups (1,723.61 cm⁻¹). The ¹³C NMR spectrum (**Figure 2**) showed the resonances of a carbonyl carbon, fifteen methine carbons, 2 methylene carbons, a methyl carbons and 6 quaternary carbon. The ¹H NMR spectrum (**Figure 2**) showed characteristic signals of H-2, H-3_{ax} and H-3_{eq} of flavanones at δ 5.51 (*dd*, 12.8, 3.0), δ 3.26 (*dd*, 17.3, 12.8) and δ 2.78 (*dd*, 17.3, 3.0). The spectrum further showed signals of a chelated hydroxyl group at δ 12.03 (5-OH), an AB pattern with *meta* coupling constant at δ 6.17 (H-6) and δ 6.16 (H-8), and *para*-substituted aromatic protons H-2'/H-6 at δ 7.40 and of H-3'/H-5' at δ 6.90. The spectrum also showed signals of a glucoside moiety at δ 5.18 (*d*, 7.5 Hz, glucosyl H-1''), δ 3.72 and 3.86 (*m*, H-6''), δ 3.41-3.98 (*m*, glucosyl protons) and rhamnoside moiety at δ 5.34 (*brm*, rhamnosyl, H-1'''), δ 3.41-3.98 (*m*, rhamnosyl protons), and δ 1.14 (*m*, rhamnosyl CH₃). The ¹H, ¹³C, DEPT 90, DEPT135, COSY, NOSEY, HSQC and HMBC experiments were performed in order to confirm the structure of 4. Furthermore, comparison NMR with data with the previously reported data, it was clear that the 4 was naringin [27].

Biological activities

The antioxidant activities and tyrosinase inhibition of compounds 1 - 4 was evaluated as show in **Table 1**. Compounds 1 - 4 showed scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) with IC₅₀ values of 0.769 ± 0.243, 0.219 ± 0.011, 0.330 ± 0.010, and 0.358 ± 0.005 mg/mL, respectively. All compounds had similar and weaker inhibitory activity in the DPPH assay (Trolox 0.006 ± 0.000 µg/mL). Coumarins and flavonoids are potential sources of natural antioxidants. In general, the increase in antioxidant properties of coumarins and flavonoids attribute the observed differences on the number and positioning of hydroxyl groups or electron donating group [28]. The results show that compounds 2 - 4 have a stronger inhibition of antioxidant activity than compound 1 because they have more hydroxyl groups or donor electrons in the structure. Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions. Kojic acid was used as a positive control. The values IC₅₀ for the compounds 1 - 4 were 4.874 ± 0.403, 0.033 ± 0.002, 0.970 ± 0.048 and 0.615 ± 3.077 mg/mL, respectively. The compounds 2 - 4 showed a strong inhibitory effect with an IC₅₀ value of less than 1.000 mg/mL, but still lower than that of kojic acid (0.003 ± 0.000 µg/mL). In contrast, the IC₅₀ value of compound 1 is the highest. Flavonoids possess the ability to inhibit tyrosinase. The hydroxyl groups at C-5, C-4', and C-5' of flavonoids formed hydrogen bonds with the amino acid residues of tyrosinase, which played a crucial role in the binding and interaction between flavonoids and tyrosinase [29]. This is consistent with the structure of the compounds 2 - 4.

Table 1 Antioxidant and Anti-tyrosinase activities of compounds 1 - 4.

Sample	DPPH IC ₅₀ (mg/mL)	Tyrosinase IC ₅₀ (mg/mL)
1	0.769 ± 0.243	4.874 ± 0.403
2	0.219 ± 0.011	0.033 ± 0.002
3	0.330 ± 0.010	0.970 ± 0.048
4	0.358 ± 0.005	0.615 ± 3.077
Trolox (µg/mL)	0.006 ± 0.000	-
Kojic acid (µg/mL)	-	0.003 ± 0.000

The experiments are not tested.

Table 2 Antibacterial activity of compounds 1 - 4.

Sample	MIC/MBC ($\mu\text{g/mL}$)	
	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922
1	> 2,048 / -	> 2,048 / -
2	256 / > 256	128 / > 256
3	256 / > 512	256 / > 512
4	> 2,048 / -	> 2,048 / -
Vancomycin	0.5 / 1	-
Amphotericin	-	2 / 8

The experiments are not tested.

Compounds 1 - 4 were tested for their antibacterial activity on *S. aureus* ATCC25923, and *E. coli* ATCC25922 as show in **Table 2**. The MIC and MBC values of compound 2 were 256 / > 256 and 128 / > 256 $\mu\text{g/mL}$ against *S. aureus* ATCC25923, and *E. coli* ATCC25922, respectively. In addition, the MIC and MBC values of compound 3 against *S. aureus* ATCC25923 were 256 / > 512 $\mu\text{g/mL}$ as same as *E. coli* ATCC25922. In contrast, compounds 1 and 4 exhibited weak antibacterial activity against *S. aureus* ATCC25923 and *E. coli* ATCC25922 with MIC > 2,048 $\mu\text{g/mL}$. Interestingly, the compounds 2 and 3 inhibited both Gram-positive and Gram-negative bacteria. The results obtained from this study indicate that the compounds might be a great source of broad-spectrum antibacterial agents. The antibacterial mechanism of compounds 2 and 3 might be presented of phenolic and flavonoid in the peel extracts of *C. grandis*, causing increased membrane permeability [30].

Compounds 1 - 4 were evaluated for cytotoxicity against cell lines MCF-7 and HT-29. The cytotoxicity of pure compounds was determined with the highest concentration at 20 $\mu\text{g/mL}$. According to the classification of cytotoxicity, the compounds 1 - 4 exhibited no inhibitory effect at a concentration of 20 $\mu\text{g/mL}$, indicating no cytotoxic effects on MCF-7 and HT-29 cells.

Conclusions

A phytochemical investigation of the extracts from the peels of *C. grandis* Linn. (Tubtim Siam Pomelo) led to the isolation of 1 coumarin (1), 2 flavonoids (2 - 3), and one flavonoid glycoside (4). They are reported from this plant for the first time. Meanwhile, compounds 1 - 4 gave good antioxidant and anti-tyrosinase activities.

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References

- [1] ZH Abudayeh, IIA Khalifa, SM Mohammed and AA Ahmad. Phytochemical content and antioxidant activities of pomelo peel extract. *Phcog. Res.* 2019; **11**, 244-7.
- [2] X Lu, C Zhao, H Shi, Y Liao, F Xu, H Du, H Xiao and J Zheng. Nutrients and bioactives in citrus fruits: Different citrus varieties, fruit parts, and growth stages. *Crit. Rev. Food Sci. Nutr.* 2023; **63**, 2018-41.
- [3] L Cebadera-Miranda, L Domínguez, MI Dias, L Barros, ICFR Ferreira, M Igual, N Martínez-Navarrete, V Fernández-Ruiz, P Morales and M Cámara. *Sanguinello* and *Tarocco* (*Citrus sinensis* [L.] Osbeck): Bioactive compounds and colour appearance of blood oranges. *Food Chem.* 2019; **270**, 395-402.
- [4] RK Saini, RK Saini, K Sharma, P Prasad, X Shang, KG Mallikarjuna and K Young-Soo. Bioactive compounds of citrus fruits: A review of composition and health benefits of carotenoids, flavonoids, limonoids, and terpenes. *Antioxidants* 2022; **11**, 239.

- [5] S Li, P Min-Hsiung, L Chih-Yu, D Tan, Y Wang, F Shahidi and H Chi-Tang. Chemistry and health effects of polymethoxyflavones and hydroxylated polymethoxyflavones. *J. Funct. Foods* 2009; **1**, 2-12.
- [6] EI Oikeh, ES Omoregie, FE Oviasogie and K Oriakhi. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. *Food Sci. Nutr.* 2015; **4**, 103-9.
- [7] L Aliberti, L Aliberti, VD Feo, LD Martino, F Nazzaro and LF Souza. Chemical composition and *in vitro* antimicrobial, cytotoxic, and central nervous system activities of the essential oils of *Citrus medica* L. cv. 'Liscia' and *C. medica* cv. 'Rugosa' Cultivated in Southern Italy. *Molecules* 2016; **21**, 1244.
- [8] S Yong-Xin, IS Manawasinghe, D Zhang-Yong, L Jie-Rong and M Luo. Diversity of endophytic bacterial populations in citrus grandis cv. tomentosa. *Chiang Mai J. Sci.* 2022; **49**, 985-98.
- [9] Z Zou, W Xi, Y Hu, C Nie and Z Zhou. Antioxidant activity of Citrus fruits. *Food Chem.* 2016; **196**, 885-96.
- [10] K Jae-Won, HC Ko, J Mi-Gyeong, SH Han, HJ Kim and K Se-Jae. Phytochemical content and antioxidant activity in eight citrus cultivars grown in Jeju Island according to harvest time. *Int. J. Food Properties* 2023; **26**, 14-23.
- [11] S Chun-Yan, J Jian-Guo, W Zhu and Q Ou-Yang. Anti-inflammatory effect of essential oil from *Citrus aurantium* L. var. amara engl. *J. Agr. Food Chem.* 2017; **65**, 8586-94.
- [12] Z Aumeeruddy-Elalfi, A Gurib-Fakim and MF Mahomoodally. Kinetic studies of tyrosinase inhibitory activity of 19 essential oils extracted from endemic and exotic medicinal plants. *S. Afr. J. Bot.* 2016; **103**, 89-94.
- [13] JS Chen, CI Wei and MR Marshall. Inhibition mechanism of kojic acid on polyphenol oxidase. *J. Agr. Food Chem.* 1991, **39**, 1897-901.
- [14] N Mallick and RA Khan. Antihyperlipidemic effects of citrus sinensis, citrus paradisi, and their combinations. *J. Pharm. BioAllied Sci.* 2016; **8**, 112-8.
- [15] L Li-Yun, H Boa-Chan, C Kuan-Chou and RY Peng. Integrated anti-hyperlipidemic bioactivity of whole citrus grandis [L.] osbeck fruits—multi-action mechanism evidenced using animal and cell models. *Food Funct.* 2020; **11**, 2978-96.
- [16] S Cirmi, A Maugeri, N Ferlazzo, S Gangemi, G Calapai, U Schumacher and M Navarra. Anticancer potential of citrus juices and their extracts: A systematic review of both preclinical and clinical studies. *Front. Pharmacol.* 2017; **8**, 420.
- [17] F Palomares-Alonso, H Jung, GC Vidal-Cantú, IS Rojas-Tomé, DF González-Esquivel, VPDL Cruz, IG Hernández, GP Hernández, F Pérez-Severiano and N Castro. Moringa oleifera extracts and praziquantel combination: Bioavailability in rats and cysticidal activity in a murine model. *Revista Brasileira Farmacognosia* 2020; **30**, 251-6.
- [18] T Mei-Lin, L Cai-Di, KA Khoo, W Mei-Ying, K Tsang-Kuei, L Wei-Chao, Z Ya-Nan and Ya-Ying Wang. Composition and bioactivity of essential oil from *Citrus grandis* (L.) osbeck 'Mato Peiyu' leaf. *Molecules* 2017; **22**, 2154.
- [19] AO Ademosun, G Oboh, TA Olasehinde and OO Adeoyo. From folk medicine to functional food: a review on the bioactive components and pharmacological properties of citrus peels. *Orient. Pharm. Exp. Med.* 2018; **18**, 9-20.
- [20] S Pichaiyongvongdee and R Haruenkit. Investigation of limonoids, flavanones, total polyphenol content and antioxidant activity in seven Thai pummelo cultivars. *Agr. Nat. Res.* 2009; **43**, 458-66.
- [21] P Sae-Lim, S Yuenyongsawad and P Panichayupakaranant. Chamuangone-enriched Garcinia cowa leaf extract with rice bran oil: Extraction and cytotoxic activity against cancer cells. *Phcog. Mag.* 2019; **15**, 183-8.
- [22] JS Lewis. *M100 Performance standards for antimicrobial susceptibility testing*. 33rd ed. Clinical and Laboratory Standards Institute, Pennsylvania, 2023.
- [23] S Sriwiriyan, T Ninpesh, Y Sukpondma, T Nasomyon and P Graidist. Cytotoxicity screening of plants of genus *Piper* in breast cancer cell lines. *Trop. J. Pharmaceut. Res.* 2014; **13**, 921.
- [24] C Bézivin, S Tomasi, DF Lohézic-Le and J Boustie. Cytotoxic activity of some lichen extracts on murine and human cancer cell lines. *Phytomedicine* 2003; **10**, 499-503.
- [25] C Riviere, L Goossens, N Pommery, C Fournau, A Delelis and JP Henichart. Antiproliferative effects of isopentenylated coumarins isolated from *Phellolophium madagascariense* Baker. *Nat. Prod. Res.* 2006; **20**, 909-16.
- [26] W Dandan, W Jian, H Xuehui, T Ying and N Kunyi. Identification of polymethoxylated flavones from green tangerine peel (*Pericarpium Citri Reticulatae Viride*) by chromatographic and spectroscopic techniques. *J. Pharmaceut. Biomed. Anal.* 2007; **44**, 63-9.

-
- [27] D Hamdan, MZ El-Readi, A Tahrani, F Herrmann, D Kaufmann, N Farrag, A El-Shazly and M Wink. Chemical composition and biological activity of Citrus jambhiri Lush. *Food Chem.* 2011; **127**, 394-403.
- [28] IV Smolyaninov, VV Kuzmin, MV Arsenyev, SA Smolyaninova, AI Poddel'sky and NT Berberova. Electrochemical transformations and anti/prooxidant activity of sterically hindered o-benzoquinones. *Russ. Chem. Bull.* 2017; **66**, 1217-29.
- [29] M Fan, H Ding, G Zhang, X Hu and D Gong. Relationships of dietary flavonoid structure with its tyrosinase inhibitory activity and affinity. *LWT* 2019; **107**, 25-34.
- [30] G Yuan, Y Guan, H Yi, S Lai, Y Sun and S Cao. Antibacterial activity and mechanism of plant flavonoids to gram-positive bacteria predicted from their lipophilicities. *Sci. Rep.* 2021; **11**, 10471.