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Chemical constituents from *Citrus grandis* Linn. and their biological activities

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Abstract

This research aimed to study the chemical constituents and their biological activity of the peels of *Citrus grandis* Linn. (Tubtim Siam Pomelo). The peels of *C. grandis* Linn. were immersed in acetone at room temperature. After evaporation, the acetone extract was purified with column chromatography (CC) to give a flavonoid (**1**). The structure of pure compound (**1**) was elucidated by analyses of spectroscopic data and comparing their spectroscopic data with the previously published data. Crude acetone and **1** have been evaluated for antioxidant, anti-tyrosinase, and antimicrobial. Compound **1** has DPPH radicals and tyrosinase with IC_{50} 0.123 ± 0.004 and 0.357 ± 0.117 mg/mL, respectively.

Keywords: *Citrus grandis* Linn, flavonoids, antioxidant activity, anti-tyrosinase activity, antimicrobial activity

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1. Introduction

Citrus is undoubtedly the most important genus in the family Rutaceae, and it is native to the tropics of South China, Vietnam, the Philippines, Thailand, India, and the Malaysian Peninsula. *Citrus* contains a rich source of secondary metabolites, such as flavonoids, acridones, limonoids, and coumarins. This genus has been used to treat many symptoms of disease in humans, such as coughs, skin inflammation, muscle pains, stomach upsets, and ringworm infections [1, 2, 3, 4]. *C. grandis* (Tubtim Siam Pomelo), grown in Pak Phanang, Nakhon Si Thammarat, Thailand, is a tree with edible fruits. The peels of pomelo fruits (*C. grandis* Linn.) are often discarded as waste. Several studies proved that pomelo byproducts contained bioactive compounds [5, 6, 7]. Chemical constituents and biological activities of extracts from pomelo peels of *C. grandis* Linn. (Tubtim Siam Pomelo) have not been reported. To understand the chemical components and to search for beneficial bioactive metabolites. In our project, compounds in peel extracts were investigated and studied for their antioxidant, anti-tyrosinase, and antimicrobial activities.

2. Materials and Methods

2.1 General experimental procedures

The ^{1}H and ^{13}C NMR spectra were recorded on an 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 performed on silica gel 60 GF254 (Merck). Column chromatography (CC) used silica gel 100 (70−230 Mesh ASTM, Merck). Thin−layer chromatography (TLC) was performed on silica gel 60 F254 (Merck). Solvents for extraction and chromatography were distilled at their boiling ranges before use.

2.2 Extraction and isolation

The dried peels of *C. grandis* (Tubtim Siam Pomelo) were collected from Amphoe Pak Phanang, Nakhon Si Thammarat, Thailand, in October 2021. The peels of *C. grandis* (Tubtim Siam Pomelo) (2.30 kg) were 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 of hexane, hexane/acetone, acetone, acetone/MeOH, and finally with pure MeOH to furnish 13 fractions (A−M). CC further fractionated fraction E (2.2315 g) with hexane: acetone (4:1, v/v) that yielded 7 subfractions (E1−E7). CC further separated Subfraction E4 (376.5 mg) with hexane: CH_2Cl_2 (7:3, v/v) to give 8 subfractions (E4A−E4H). CC further separated Subfraction E4F (76.4 mg) with hexane:CH_2Cl_2: EtOAc (7:1:1, v/v) to give a yellow solid **1** (4.3 mg).

2.3 Evaluation of the antioxidant activity of the pure compound and Crude acetone

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 designed by dissolving 1 mg of crystalline Trolox in 1 mL 95% methanol. The DPPH assay followed the modified method [8]. 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 two independent trials.

2.4 Evaluation of Anti-tyrosinase activity of the pure compound and Crude acetone

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The assay was carried out on a 96-well microtiter plate. In each well, 40 μL of pure compounds, 80 μL of phosphate buffer (0.1 M, pH 6.8), 40 μL of tyrosinase enzyme (60 units/mL), and 40 μL 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: Tyrosinase inhibition (%) = (Absorbance of test / Absorbance of control) × 100

2.5 Evaluation of the antibacterial activity of pure compound and Crude acetone

Reference strains, including Gram-positive bacteria (*S. aureus* ATCC 25923) and Gram-negative bacteria (*Escherichia coli ATCC 25922*), were used in the study. Bacterial strains were subcultured 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 (CLSI-M27-A3) [9]. The 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 μg/mL. The microbial suspensions were adjusted to a 0.5 McFarland standard at OD 600 nm in a normal saline solution. The tested pathogens were added to the 96-well plate and incubated at 37 ºC for 18 h. MIC values were determined as the lowest gold nanoparticle concentration inhibiting microbial growth. Minimum bactericidal concentration (MBC) values were defined as the lowest concentration of the gold nanoparticles that showed no colony growth on the culture medium.

2.6 Statistical analysis

All experiments were carried out in triplicates. Data are expressed as mean ± standard deviation. Results were tested by the analysis of variance (ANOVA), and Dunnett's test was used to compare means. $P < 0.05$ was required for a statistically significant difference.

3. Results and Discussion

Extraction and purification of the acetone extract from the peels of *C. grandis* Linn. resulted in isolated known compounds (**1**) (Fig. 1). Analyses of spectroscopic data analysis elucidated the structure of pure compounds.

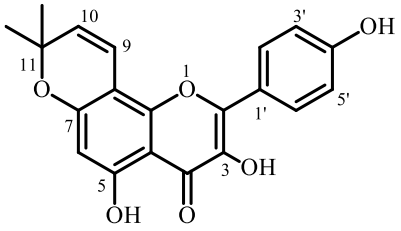

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Fig. 1 Structure of compound 1 was isolated from *C. grandis* Linn**.**

Compound **1** was obtained as a yellow solid. The ^{13}C NMR spectrum (**Table 1.**) showed the resonances of a carbonyl carbon at δ 175.4 (C-4) of flavonoid moiety. The ^{1}H NMR spectrum **(Table 1.)** showed a chelated hydroxyl group (δ 11.79,

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5-OH), a *para*-substituted B ring at δ 8.13 (*d*, 9.0, H-2'/H-6'); δ 6.99 (*d*, 9.0, H-3'/H-5'), a singlet hydroxy proton (δ 6.60, 3-OH, *s*), a singlet aromatic proton (δ 6.29, H-6) and a 2,2-dimethyl chromene ring (δ 1.56, *s*, H-12; 6.81, *d*, 10.0, H-9; 5.61, *d*, 10.0, H-10). A singlet aromatic proton at δ 6.25 was confirmed for H-6 due to the correlations of 5-OH to C-6 (δ 99.7) and C-4 (δ 101.5), and H-6 to C-4a. The chromene ring was attached at C-7 and C-8 of the A ring due to the H-6 and H-10 showing a correlation to C-8 (δ 103.8) and H-9 to C-7 (δ 159.5). Finally, the flavonol was proposed to complete the structure with the singlet hydroxyl group resonating at δ 6.60 and the carbon signal resonating at δ 135.8 (C-3). The ^{1}H, ^{13}C, H-H COSY, HSQC, and HMBC experiments were performed to confirm the structure **1**. Furthermore, comparing NMR with data with the previously reported data, it was clear that the **1** was citrusinol [10].

Table 1 ^{1}H and ^{13}C NMR of compound **1**

Position	δ_H **(multiplicity)**	δ_C **(C-Type)**
2	-	145.3 (C)
3	-	135.8 (C)
4	-	175.4 (C=O)
4a	-	101.5 (C)
5	-	160.6 (C)
6	6.28 (*s*)	99.7 (CH)
7	-	159.9 (C)
8	-	103.8 (C)
8a	-	151.2 (C)
9	6.81 (*d*, 10.0)	114.8 (CH)
10	5.61 (*d*, 10.0)	127.4 (CH)
11	-	77.1 (C)
12	1.56 (*s*)	28.3 (CH_3)
1'	-	123.8 (C)
2'/6'	8.13 (*d*, 9.0)	129.6 (CH)
3'/5'	6.99 (*d*, 9.0)	115.7 (CH)
4'	-	157.5 (C)
3-OH	6.60 (*s*)	-
5-OH	11.79 (*s*)	-

The antioxidant and tyrosinase activities of crude acetone and pure compound were evaluated. Compounds **1** and crude acetone showed the scavenging DPPH radicals with IC_{50} 0.123 ± 0.004 and 2.874 ± 0.078 mg/mL, respectively, which indicate that they have weak activity compared to Trolox **(**0.006 ± 0.000 μg/mL**)**. Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions. Kojic acid was used as a positive control. The value IC_{50} for compound **1** was 0.357 ± 0.117 mg/mL, whereas IC_{50} for the crude acetone was 3.580 ± 0.847 mg/mL.

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Table 2 Antioxidant and anti-tyrosinase activities of **1** and crude acetone

Sample	IC_{50} (mg/mL)	
	Antioxidant activity	**Antityrosinase activity**
1	0.123 ± 0.004	0.357 ± 0.117
Crude acetone	2.874 ± 0.078	3.580 ± 0.847
Trolox (μg/mL)	0.006 ± 0.000	
Kojic acid (μg/mL)		0.003 ± 0.000

Crude acetone and compound **1** were tested for their antibacterial activity on *S. aureus* ATCC25923 and *E. coli* ATCC25922, as shown in **Table 3**. They had MIC/MBC in the range of 1024–>2048 μg/mL**.**

Table 3 Antibacterial activity of **1** and crude acetone

Sample	MIC/MBC (μg/mL)	
	***Staphylococcus aureus* ATCC 25923**	***Escherichia coli* ATCC 25922**
1	1024/>1024	1024/>1024
Crude acetone	2048/>2048	>2048/-
Vancomycin	0.5/1	-
Amphotericin	-	2/8

Previously reported, flavanones, acridones, coumarins and limonoids were identified from *C. grandis*, including naringin, eriocitrin, neoeriocitrin, narirutin, neohesperidin, hesperidin, didymin, poncirin, quercetin acacetin, rutin, tangeretin, cosmosiin, diosmetin, diosmin [7, 11], 5-hydroxyacronycine, acriginine A, atalafoline, baiyumine A-B, buntanine, buntanmine, grandisine I & II, pumiline, honyumine, natsucrin, prenyl citpressine, citropone A- B, glycocitrine [12], 5-geranoxy-7-methoxy-coumarin, aurapte, auraptene, bergamottin, 5-methoxy seselin , 5-methyltodannol, 6-hydroxymethylherniarin [13], limonin, nomilin [7]. In this work, the chemical constituents from the peels of *C. grandis* **(**Tubtim Siam Pomelo**)** were present in citrusinol (1).

4. Conclusions

A phytochemical investigation of the extracts from the peels of *C. grandis* Linn (Tubtim Siam Pomelo) led to the isolation of one flavonoid. It was reported from this plant for the first time.

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