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RESEARCH ARTICLE

Phytochemical Analysis of *Citrus grandis* (Tubtim Siam) Leaves and Peels Extracts and Their Biological Potential

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Abstract Pomelo (*Citrus grandis*) is renowned for its sweet, tangy flavor, with the Tubtim Siam cultivar being especially popular in Thailand for its vibrant reddish-pink flesh. While citrus leaves and peels are typically discarded, they may contain valuable bioactive compounds. This research examines the phytochemical profile, antioxidant, antimicrobial, and anticancer properties of *C. grandis* (Tubtim Siam) extracts obtained from its leaves and peels. Phytochemical screening revealed the presence of alkaloids, flavonoids, terpenoids, and coumarins in all extracts, with coumarins absent in the water extract of leaves (LE) and anthraquinones undetected in all samples. The antioxidant activity, evaluated through the DPPH radical scavenging assay, revealed that the ethanolic peel extract (PE) exhibited the strongest antioxidant potential (IC_{50} 0.532 ± 0.02 mg/mL), correlating with high levels of total phenolics (96.71 ± 2.04 mg GAE/g) and flavonoids (958.06 ± 63.28 mg QE/g). The extracts exhibited limited antimicrobial and anticancer effects. GC-MS analysis of the PE extract identified 22 bioactive compounds, including naringenin (21.37%), meranzin hydrate (11.17%), isoauraptene (5.39%), D-limonene (2.01%), and elemicin (1.64%). Notably, this study identified six compounds newly reported in Tubtim Siam extracts through GC-MS analysis, including 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one, 4-(1-aminoethyl)phenol, limonene glycol, *p*-methoxytodadiol, 4-(1*E*)-3-hydroxy-1-propenyl-2-methoxyphenol, and *N*-(4-methoxyphenethyl)-benzamide. These findings highlight the potential of *C. grandis* as a valuable reservoir of biologically active compounds, supporting its therapeutic applications and emphasizing the need for further research into its bioactive properties.

Keywords: *Citrus grandis*, phytochemicals, phenolic compounds, antioxidant activity, GC-MS analysis.

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Introduction

Plant-based natural products have long been integral to traditional medicine and remain a focal point of scientific research due to their broad therapeutic potential. These substances possess a variety of biological activities that play an important role in promoting human health. Among them, natural antioxidants have garnered particular attention for their ability to mitigate oxidative stress, a major contributor to aging and the development of chronic diseases such as cancer, diabetes, and cardiovascular disorders [1]. Flavonoids and phenolic compounds, abundant in many plants, are known to neutralize free radicals, regulate enzyme activities, and modulate signaling pathways involved in inflammation and apoptosis [2]. In parallel, the antimicrobial properties of plant-derived extracts provide

promising alternatives to conventional antibiotics, particularly in light of rising antimicrobial resistance. Phenolic compounds, for instance, can disrupt bacterial cell walls, inhibit microbial enzymes, and interfere with nucleic acid synthesis [3]. The Citrus genus is globally recognized for its desirable flavor and abundant nutritional and bioactive constituents. Citrus fruits are particularly rich in a diverse array of bioactive compounds, including phenolic compounds—such as flavonoids, phenolic acids, and coumarins—as well as terpenoids like limonoids and carotenoids, which contribute to their health-promoting properties [4]. One notable example is *Citrus grandis*, widely known as pomelo, which belongs to the Rutaceae family. Pomelo is primarily cultivated in tropical and subtropical regions, with Asia being a key area of production. Thailand, in particular, is renowned for its diverse pomelo cultivars, such as Kao Hom, Kao Pan, Kao Yai, Kao Tangkwa, Kao Numpueng, Pattavee, Tha Knoi, Tong Dee, and Tubtim Siam. These cultivars are recognized for their abundant bioactive compounds, particularly flavonoids, which vary significantly across different varieties. Pomelo has traditionally been employed in folk medicine to address various health concerns, such as digestive disorders, respiratory ailments, and skin infections [5-6]. Beyond its nutritional value, pomelo has garnered scientific interest for its rich phytochemical composition, encompassing essential oils, phenolic acids, flavonoids, alkaloids, and coumarins—compounds recognized for their diverse therapeutic properties [7-8]. Phenolics and flavonoids are bioactive compounds widely recognized for their antioxidant, anti-inflammatory, anticancer, and antimicrobial effects [9-12]. Extensive research has been conducted to explore the phytochemical composition and biological activities of pomelo extracts. Previous studies have highlighted the biological potential of different parts of the pomelo fruit. Among pomelo cultivars, Thong Dee has shown the highest polyphenol content and antioxidant activity in the flavedo and seeds, while Tha Knoi demonstrated similar properties in the albedo and segment membranes. Kao Tangkwa was notable for its antioxidant activity in seeds [13]. Several studies have investigated the biological effects of pomelo. Naradisorn and Ruenkum (2009) examined the antimicrobial activity of crude dichloromethane extracts from pomelo albedo against *Colletotrichum gloeosporioides*. The extracts, tested at concentrations of 25%, 50%, 75%, and 100%, showed significant antimicrobial effects [14]. Additionally, Buachan *et al*. (2014) reported the presence of natural antioxidants in freeze-dried pomelo fruit extract powder. Their study highlighted the extract's potential to promote cell migration and slow down cellular aging [15]. Among, six pomelo cultivars, including Kao-Yai, Thong-dee, Kao-Tangkwa, Kao-Numpueng, Ta-Koi, and Tubtim Siam all exhibited notable antioxidant and antihyperlipidemic properties [16]. Tubtim Siam cultivar has emerged as particularly promising. Recent work by Balmori *et al*. (2023) demonstrated that fermentation of Tubtim Siam pomelo juice significantly enhanced its functional properties, resulting in the highest levels of total polyphenols, flavonoids, and key flavonoids such as naringin and naringenin compared to other cultivars [17].

The Tubtim Siam cultivar was specifically selected due to its local economic importance, unique pigmentation, and reported ethnomedicinal use in Nakhon Si Thammarat, Thailand, yet its phytochemical and pharmacological profiles remain underexplored in the scientific literature. Despite its commercial value, comprehensive studies on the phytochemical and bioactive properties of its non-edible parts—particularly leaves and peels—are limited. Leaves and peels are known to accumulate flavonoids, coumarins, and essential oils. Utilizing these parts can also support waste valorization in agro-industries. The selection of only peels and leaves in this study is intentional, based on their phytochemical richness, availability, and relevance to sustainable resource use. Moreover, previous studies have not systematically compared solvent effects (ethanol vs. water) on extraction yield and bioactivity in this cultivar. Solvent selection plays a crucial role in the extraction and recovery of bioactive compounds from plant matrices. Ethanol and water are two commonly used solvents, each offering distinct advantages based on their polarity and safety profiles. Ethanol is effective in extracting a broad spectrum of moderately polar to non-polar compounds, including flavonoids and coumarins, whereas water, a highly polar solvent, is often employed for extracting hydrophilic phenolics and other polar phytochemicals. Comparative studies on solvent efficiency can provide critical insights into optimizing extraction processes for maximal bioactivity and yield. This study addresses this gap by comparing ethanol and water extracts to determine which solvent more effectively captures the bioactive potential of the *C. grandis* ‘Tubtim Siam’ cultivar. Notably, the investigation identified several previously unreported compounds in this variety. These findings enhance understanding of the bioactive constituents of *C. grandis* ‘Tubtim Siam’ and support its potential for developing health-promoting and functional foods to meet the growing demand for natural therapeutic alternatives.

Materials and Methods

Material

The leaves and peels of *C. grandis* cultivar "Tubtim-Siam" were collected in July 2021 from plants cultivated as part of the agricultural development program at the Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhon Si Thammarat Province, Thailand, for use in student teaching. The collected leaves and peels were washed under running water, drained, dried until completely dehydrated at 60°C in a convection oven. After being finely chopped, the samples were stored in vacuum-sealed bags for subsequent testing in the next step. Breast cancer (MCF-7), lung cancer (A549) and colon cancer (HT-29) cell lines (American Type Culture Collection, USA), RPMI-1640 medium (Gibco, USA). The research's chemical and reagent supplies came from Sigma-Aldrich and Merck.

Instrumentation

UV Spectrophotometer (Libra Biochrome, UK), CO_2 Incubator (SHELDON, USA), Microplate Reader (San Jose, CA, USA), Rotary Evaporator (Buchi, Switzerland), Gas chromatography-mass spectrometer (Agilent, USA).

Preparation of Plant Extracts

The dried peels of *C. grandis* (200 g) were finely chopped and macerated in 2.5 L of 99% ethanol at room temperature. After solvent evaporation, 12.4 g of a dark-brown gum were obtained, representing a yield of 6.2%. Similarly, dried leaves of (200 g) were chopped and soaked in 2.5 L of 99% ethanol under the same conditions, yielding 13.13 g of dark-brown gum, with a yield of 6.57%. In a parallel experiment, the dried peels (200 g) were immersed in 2.5 L of deionized water (DI water) at room temperature, resulting in 9.3 g of dark-brown gum following solvent evaporation, corresponding to a yield of 4.65%. The dried leaves (200 g) were also treated with 2.5 L of DI water at room temperature, yielding 9.09 g of dark-brown gum, with a yield of 4.55%.

Phytochemical Determination of *C. grandis* Extracts

Standard techniques were used to analyze the phytochemical constituents of various *C. grandis* extracts. The detection of alkaloids, flavonoids, coumarins, terpenoids, and anthraquinone was performed as previously described. In the qualitative analysis, the absence was indicated as negative (-) and the presence as positive, (+), as well as the intensity of the characteristic color.

Alkaloids: 2 mL of *C. grandis* extracts and 1 mL of Dragendorff's reagent were mixed. An orange-red precipitate's presence verified a successful outcome [18–19].

Flavonoids: Flavonoids was determined using the NH_4OH test. A few drops of 10% ammonium hydroxide were added to 3 mL of *C. grandis* extracts. The appearance of yellow fluorescence indicated a positive reaction [18–19].

Coumarins: 3 mL of 10% w/v sodium hydroxide solution was combined with 2 mL of the aqueous *C. grandis* extracts. A yellow coloration suggested the presence of coumarins [18–19].

Triterpenoids: 1 mL of 2% hydrochloric acid, 1 mL of Dragendorff's reagent, and 1 mg/mL of *C. grandis* extracts were combined. The presence of terpenoids was indicated by the emergence of a reddish-brown tint [18–19].

Anthraquinones: The *C. grandis* extracts solution was combined with 5 mL of diluted sulfuric acid, heated for 5 minutes, and subsequently filtered. The filtrate was extracted with dichloromethane (CH_2Cl_2), and the two layers were separated. Five drops of 10% ammonium hydroxide were added to the CH_2Cl_2 layer, shaken, and observed. A pink color confirmed the presence of anthraquinones [18–19].

Quantification of total phenolic content (TPC): 5 mL of the *C. grandis* extract (1 mg/mL) or distilled water was mixed with 0.5 mL of a 10% Folin-Ciocalteu reagent, mixed well and incubated in the dark for 7 minutes. Then 1 mL of a 10% Na_2CO_3 solution was added, mixed well and the mixture was incubated at room temperature for 30 minutes. The absorbance of the resulting solution was then determined at a wavelength of 750 nm using a UV-VIS spectrophotometer [20]. Gallic acid was used as the standard reagent. The mean absorbance value was used to calculate the TPC and expressed as mg GAE/g extract [20].

Quantification of total flavonoids content (TFC): TFC was determined according to the method described by Biju *et al*. (2014). A 0.35 mL portion of the sample solution with a concentration of 1 mg/mL

was combined with 0.15 mL of a 5% $NaNO_2$ solution (w/v) and incubated for 5 minutes. Next, 0.15 mL of a 10% $AlCl_3$ solution in ethanol and then 0.35 mL of a 1 M NaOH solution were added. The mixture was shaken well and incubated for 5 minutes. The absorbance of the resulting solution was measured at a wavelength of 510 nm [21]. The TFC was quantified by comparing the absorbance with a standard calibration curve of quercetin solution whose concentrations ranged from 0 to 150 µg. The results are given in mg QE/g extract [21].

Evaluation of Antioxidant Activity of *C. grandis* Extracts

The extract's 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity was assessed using a revised method based on Blois (1958). The reaction involved mixing a 0.3 mM DPPH solution with sample solutions of different concentrations (ranging from 0 to 1 mg/mL). The combination was kept in the dark at room temperature for 30 minutes. The absorbance was recorded at a wavelength of 517 nm utilizing a spectrophotometer, with ethanol serving as the reference (blank) [22]. The percentage of inhibition was determined using this formula:

$$\%\ inhibition = \frac{(A_{control} - A_{sample})}{A_{control}} x100$$

Where $A_{control}$ and A_{sample} denote the absorbance readings of the reaction absent and present with the sample, respectively. The IC_{50} value, representing the concentration that inhibited 50% of the DPPH radical, was established by graphing a curve with concentrations on the x-axis and % inhibition on the y-axis, and finding the value through linear regression. Butylated hydroxytoluene (BHT) served as a positive control.

Antimicrobial Activity *C. grandis* Extracts

On Mueller-Hinton agar plates, *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were subcultured for the entire night at 37°C. The minimum inhibitory concentration (MIC) of the *C. grandis* extract was determined according to the protocols established by the Clinical and Laboratory Standards Institute [23-24]. A dual broth microdilution technique was utilized, with the *C. grandis* extract and antibiotics being prepared in Mueller-Hinton broth within a sterile 96-well plate, reaching final concentrations ranging from 5 to 80 µg/mL. The bacterial suspensions were modified to a 0.5 McFarland standard with normal saline at an optical density of 600 nm. After being added to the wells, the pathogens were cultured for eighteen hours at 37°C. The lowest concentration of the extract that completely stopped microbial growth was determined to be the MIC. The extracts showing antimicrobial properties at a concentration of 200 µg/mL were further assessed to find their MIC through a two-fold serial dilution technique, with concentrations varying from 0.25 to 128 µg/mL. The MIC was identified as the minimal concentration of the extract that entirely inhibited microbial growth. Wells showing inhibition were streaked onto nutrient agar for bacterial strains and incubated under suitable conditions. Extracts exhibiting MIC values below 10 µg/mL were deemed to possess robust antimicrobial activity.

Anticancer Activity of *C. grandis* Extracts

RPMI-1640 media was used to cultivate MCF-7, A549, and HT-29 cell lines. 10% fetal bovine serum and 1% penicillin/streptomycin mixture were added, and the cells were kept at 37°C in a humidified incubator with 5% CO_2. After the cells attained 80–90% confluence, they were separated with a 0.25% trypsin-EDTA, and cell count was evaluated using a hemocytometer. To evaluate cell viability following *C. grandis* extracts treatment, an MTT assay was conducted based on mitochondrial function. The cells were inoculated at a density of 1 × 10^4 cells per well in 96-well plates (Corning) and left to incubate for 24 hours in a humidified chamber. The *C. grandis* extracts were introduced at concentrations between 0 to 500 µg/mL, and the cells underwent treatment for 72 hours. Following treatment, MTT solution was added to each well at a final concentration of 0.5 mg/mL, and the cells were incubated at 37°C (1 hr) in a 5% CO_2 incubator. Dimethyl sulfoxide was subsequently added, and the plates were incubated at ambient temperature (30 min). A microplate reader was used to measure the resultant solution's absorbance at 570 and 650 nm [25]. The IC_{50} value was determined.

Analysis Using Gas Chromatography-Mass Spectrometry (GC-MS) Extract from *C. grandis*

The chemical composition of the *C. grandis* extract was analyzed using a 7890 B GC–5977C MSD system (Agilent Technologies, Inc., USA). Separation was achieved with an HP-5MS UI column (30 m x 0.25 x 0.25 mm). The gas chromatography assessment was conducted in splitless injection mode, at 250°C and using a 1.0 µL injection volume. The oven began at a temperature of 50°C (5 min), then slowly increased to 250°C (15 min) at a speed of 5°C each minute. Mass spectrometry detection was performed using electron ionization (EI) at 70 eV. The temperatures for the interface, MS quadrupole, and ion source were adjusted to 250°C, 230°C, and 150°C, respectively. The mass scanning range was

set from 30 m/z to 500 m/z, including a solvent delay of 5 minutes. The identification of compounds was conducted by contrasting the obtained mass spectra with those found in the NIST23 and Wiley 23 databases. The relative abundance of the identified compounds from the GC-MS analysis was calculated as the percentage of each compound's peak area divided by the total peak area of all identified compounds in the same sample.

Analysis of Statistics

SPSS for Windows (version 16.0) was used for data analysis. With n = 3, the results are displayed as mean ± standard deviation (SD). The threshold for statistical significance was set at $P < 0.01$.

Results and Discussion

This study explored the phytochemical composition, as well as the antioxidant, antimicrobial, and anticancer activities, of *C. grandis* (Tubtim Siam) peels and leaves, utilizing ethanol and water as solvents. The extraction efficiency of bioactive compounds from *C. grandis* was affected by the solvent used. The ethanol extract of the leaves (LE) showed the highest yield of 6.57%, followed by the ethanol extract of the peels (PE) with 6.20%. Water extracts from the peels (PW) and leaves (LW) yielded 4.65% and 4.55%, respectively. These differences in extraction yield are attributed to the varying polarity of the solvents, the structural characteristics of the plant matrix, and the solubility of the bioactive compounds. Ethanol, with its intermediate polarity, is effective at extracting both polar and non-polar secondary metabolites, explaining the higher yields observed in LE and PE. In contrast, water, being highly polar, is less efficient at extracting less polar compounds, resulting in lower yields for PW and LW. Furthermore, the slightly higher yield of the ethanolic leaf extract compared to the peel extract may reflect a greater abundance or higher solubility of extractable secondary metabolites in the leaf tissue. These findings provide a critical foundation for interpreting the subsequent biological activity results.

Chemical Composition

Phytochemical screening of the *C. grandis* (Tubtim Siam) extracts revealed the presence of various compounds, as summarized in Table 1. Alkaloids, flavonoids and terpenoids were consistently detected in all extracts, while coumarins were absent in the water extract of leaves (LW) and anthraquinones were not detected in all samples. The absence of coumarins in LW suggests that the choice of solvent significantly influences the extraction of certain compounds. Ethanol appears to be a more efficient solvent for the extraction of a broader range of secondary metabolites, as evidenced by the presence of coumarins in ethanolic extracts (LE and PE). This observation is consistent with the finding that the moderate polarity of ethanol facilitates the extraction of various phytochemicals, including non-polar and slightly polar compounds.

Previous studies have shown that alkaloids, coumarins, flavonoids, and terpenoids are widely distributed in various parts of pomelo, including the stem, flower, fruit, peel, root, leaves, and bark [26-27]. In agreement with other studies, the ethanol extract of *C. grandis* peels predominantly contains flavonoids, both as aglycones and glycosides. Xi *et al*. (2014) reported that the flavonoid composition and antioxidant activities of pomelo varieties, demonstrating that the peel extracts consist mainly of flavonoids such as naringin, hesperidin, and narirutin [28]. Similarly, Nogata *et al*. (1994) confirmed the presence of naturally occurring flavonoids in citrus fruits using high-performance liquid chromatography [29]. Zhao *et al*. (2019) further elaborated on the coumarins isolated from C*. grandis* peel extracts, including compounds such as bergamottin, umbelliferone, and auraptene, which were found in the methanol and ethyl acetate fractions [30]. These compounds have been shown to exhibit anti-inflammatory effects, further reinforcing the medicinal potential of *C. grandis* peel extracts. The consistent detection of these metabolites across the extracts underscores the therapeutic potential of *C. grandis*. Notably, the absence of anthraquinones across all samples might reflect their limited biosynthesis in this variety or the need for alternative extraction methods to detect them. Overall, the findings highlight the influence of solvent polarity on phytochemical extraction and reinforce the importance of solvent selection in maximizing the yield of bioactive compounds for subsequent biological and pharmacological studies.

Table 1. Phytochemical screening of *C. grandis* (Tubtim Siam)

Sample	Phytochemical composition				
	Alkaloids	Flavonoids	Coumarins	Terpenoids	Anthraquinones
LE	+	+	+	+	-
LW	+	+	-	+	-
PE	+	+	+	+	-
PW	+	+	+	+	-

* + = Present ; - =Absent ; ethanoic extract of leaves (LE), water extract of leaves (LW), ethanoic extract of peels (PE), water extract of peels (PW)

Quantification of Total Phenolic and Flavonoid Content (TPC and TFC)

The TPC and TFC of different extracts of *C. grandis* are summarized in Table 2, showing significant effects of solvent type and plant part on these bioactive compounds. The PE exhibited the highest TPC and TFC values with 96.71±2.04 mg GAE/g and 958.06±63.28 mg QE/g, respectively, emphasizing the efficiency of ethanol in the extraction of phenolic and flavonoid compounds. In contrast, the PW showed significantly lower TPC (46.85±0.57 mg GAE/g) and TFC (3.51±0.02 mg QE/g), indicating that water has limited ability to solubilize these compounds from the peels. A contrasting pattern was observed in the leaf extracts. The LW had a slightly higher TPC (51.55±0.42 mg GAE/g) than the LE with 44.88±0.94 mg GAE/g. However, the LE had a significantly higher TFC (651.75±101.50 mg QE/g) than the LW (124.72±6.35 mg QE/g). These differences suggest that ethanol preferentially extracts flavonoids from the leaves, while water may extract certain phenols more effectively.

Previous studies on TPC and TFC in *C. grandis* have highlighted variability across plant parts, and extraction methods, depending on the cultivar, environmental conditions, soil characteristics, and the fruit's stage of maturity [31-32]. Ding *et al*. (2013) reported TPC values for pomelo peels ranging from 42.79 to 54.56 mg GAE/g and TFC values between 13.43 and 26.70 mg rutin/g [33]. Similarly, Toh *et al*. (2013) found that the TPC of pomelo pulp and peel ranged from 61.72 to 406.65 mg GAE/100 g fresh weight (FW), while the TFC in the peel varied from 13.06 ± 1.31 to 356.95 mg QE/100 g FW [34]. Chang and Azrina (2017) reported TPC values in various pomelo tissues: albedo (63.11 ± 5.03 mg GAE/g FW), flavedo (38.67 ± 4.27 mg GAE/g FW), and segment membrane (30.66 ± 1.29 mg GAE/g FW). In terms of TFC, the albedo exhibited the highest concentration (45.61 ± 5.01 mg QE/g FW), followed by the flavedo (28.05 ± 6.31 mg QE/g FW) and the segment membrane (27.16 ± 5.05 mg QE/g FW) [35]. In pomelo juice, Nishad *et al*. (2018) and Jain *et al*. (2017) observed TPC values ranging from 22.18 to 48.0 mg GAE/100 mL and approximately 55.0 mg GAE/100 mL, respectively, using 80% methanol extraction [36-37]. Focusing on the Tubtim Siam cultivar, Mäkynen *et al*. (2013) reported a relatively high TPC of 107.23 ± 0.62 mg GAE/g extract from pomelo pulp, while other cultivars such as Kao-Yai, Thong-Dee, Kao-Tangkwa, Kao-Numpueng, and Ta-Koi exhibited TPC values ranging from 101.32 to 115.02 mg GAE/g when extracted with aqueous methanol [16]. In contrast, Chooklin C. S. and Chooklin S. (2021) reported a much lower TPC of 13.67 ± 0.52 mg GAE/g dry weight from Tubtim Siam peels under optimized extraction conditions using 35% ethanol, a 1:2 peel-to-solvent ratio, and a 30-minute extraction time [38].

In the present study, the peel ethanol extract (PE) exhibited a high TPC of 96.71 ± 2.04 mg GAE/g, aligning closely with the higher values reported in previous studies, along with an exceptionally high TFC of 958.06 ± 63.28 mg QE/g. The peel water extract (PW) displayed a TPC of 46.85 ± 0.57 mg GAE/g and a markedly lower TFC of 3.51 ± 0.02 mg QE/g. Regarding the leaf extracts, the ethanol extract (LE) and water extract (LW) recorded TPC values of 44.88 ± 0.94 and 51.55 ± 0.42 mg GAE/g, respectively. However, the LE demonstrated a significantly higher TFC (651.75 ± 101.50 mg QE/g) compared to the LW (124.72 ± 6.35 mg QE/g). Overall, these findings emphasize the significant influence of plant part and extraction solvent on the recovery of phenolic and flavonoid compounds, with ethanol proving to be a particularly effective solvent, especially for pomelo peels. The exceptionally high TFC observed in the PE and LE extracts, compared to previous reports, may be attributed to differences in extraction conditions, plant maturity, or specific environmental factors affecting the Tubtim Siam cultivar. Importantly, the high phenolic and flavonoid content identified in this study suggests strong potential for these extracts to serve as natural antioxidants in nutraceutical and pharmaceutical applications.

Table 2. Total phenolic and total flavonoid contents of *C. grandis* (Tubtim Siam)

Sample	TPC (mgGAE/gram)	TFC (mgQE/gram)
LE	44.88±0.94	651.75±101.50
LW	51.55±0.42	124.72±6.35
PE	96.71±2.04	958.06±63.28
PW	46.85±0.57	3.51±0.02

Antioxidant Activity

In this study, the antioxidant activity of the Tubtim Siam cultivar of *C. grandis* was examined, focusing on ethanol and water extracts from both the peel and leaves. The DPPH radical scavenging assay was employed to assess antioxidant potential, with results expressed as IC_{50} values (Table 3). The PE showed the strongest antioxidant activity among the natural extracts, with an IC_{50} value of 0.532±0.02 mg/mL. The PW also showed a remarkable antioxidant capacity with an IC_{50} value of 0.808±0.03 mg/ml. In comparison, the LW had moderate antioxidant activity, with an IC_{50} of 1.11±0.03 mg/ml. The LE had the highest IC_{50} value among the extracts at 3.44±0.09 mg/ml, indicating the lowest antioxidant activity in this study. The synthetic antioxidant BHT (butylated hydroxytoluene) had a remarkably low IC_{50} value of 0.002±0.00 mg/ml, demonstrating the highest free radical scavenging ability of all samples tested. Phenolic compounds are recognized for their capacity to scavenge free radicals by donating hydrogen or electrons, which contributes significantly to antioxidant activity. Flavonoids, a subclass of phenolic compounds, enhance this effect by scavenging various free radicals, chelating metal ions and modulating antioxidant enzyme systems. Of the extracts tested in this study, PE had the highest TPC of 96.71 ± 2.04 mg GAE/gram and the highest TFC of 958.06 ± 63.28 mg QE/gram. These high levels of TPC and TFC correlated with the lowest IC_{50} value (0.532 ± 0.02 mg/ml), indicating its strong antioxidant activity. In contrast, the LE with the lowest TPC (44.88 ± 0.94 mg GAE/gram) and TFC (651.75 ± 101.50 mg QE/gram) had the highest IC_{50} value (3.44 ± 0.09 mg/ml), indicating weaker antioxidant activity.

Previous studies have highlighted the antioxidant potential of *C. grandis* as measured by the DPPH radical scavenging assay, with considerable variation depending on the plant part, cultivar, and extraction method used. Pichaiyongvongdee *et al*. (2014) evaluated the antioxidant activity in various tissues of seven Thai pomelo cultivars—Kao Numpueng, Thong Dee, Kao Paen, Kao Yai, Tha Knoi, Pattavee, and Kao Tanggwa—and reported the highest DPPH scavenging activity in the seeds of all cultivars, ranging from 79.97% to 85.34% inhibition [13]. In terms of IC_{50} values, variability among different extracts and plant parts has been observed. Jiang *et al*. (2014) investigated the antioxidant properties of flavonoid extracts from *C. grandis* peels (Hua Ju Hong) and found dose-dependent DPPH radical scavenging activity within a concentration range of 0.24–1.2 mg/mL [39]. Sajid *et al*. (2016) reported an IC_{50} of 332.64 µg/mL for the fruit extract of *C. grandis* [40], while a significantly lower IC_{50} value of 1.70 ± 0.00 µg/mL was reported by Ali *et al*. (2019) for the methanolic extract of pomelo, indicating a strong antioxidant capacity [9]. Focusing on the Tubtim Siam cultivar, ultrasound-assisted extraction of pomelo peels yielded an antioxidant activity with a DPPH free radical scavenging capacity of 32.58% [38]. Compared to previous studies, the present findings reveal both similarities and notable differences in the antioxidant activity of *C. grandis* extracts. Consistent with earlier reports, our results confirm that the peel exhibits stronger DPPH radical scavenging activity than the leaves, aligning with the conclusions of Jiang *et al*. (2014) and Ali *et al*. (2019), who identified the peel as a rich source of antioxidants. However, the IC_{50} value of the PE in this study was higher—indicating weaker activity—than that of the methanolic peel extract reported by Ali *et al*. (2019), but lower than that of the fruit extract reported by Sajid *et al*. (2016). This suggests that while the antioxidant potential of the Tubtim Siam peel is considerable, it may be slightly reduced when extracted with ethanol compared to more polar solvents like methanol. Nevertheless, the ethanol-extracted peel still showed markedly higher antioxidant activity than the leaf extracts (LE and LW), and remains within the effective range for natural antioxidants. These variations are likely due to differences in cultivar, solvent polarity, and extraction method.

Table 3. Antioxidant and antimicrobial activities of *C. grandis* (Tubtim Siam)

Sample	IC_{50} (mg/ml)	MIC (µg/mL)	
		S. aureus ATCC 25923	*E. coli* ATCC 25922
LE	3.44±0.09	>4,096	>4,096
LW	1.11±0.03	>4,096	>4,096
PE	0.532±0.02	>4,096	>4,096
PW	0.808±0.03	2,048	>4,096
BHT	0.002±0.00	-	-
Vancomycin	-	0.5	-
Gentamicin	-		0.25

Antimicrobial Activity

The MIC values of various *C. grandis* extracts against *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 are presented in Table 3. A lower MIC value indicates stronger antimicrobial activity, as it represents the smallest concentration of the extract needed to inhibit microbial growth. According to their high MIC values, none of the extracts tested exhibited significant antibacterial action against *S. aureus* or *E. coli*. Both the ethanoic acid and water extract of the leaves (LE and LW) showed MIC values of >4.096 µg/mL against both bacterial strains, indicating low or negligible antibacterial activity. The PE also showed MIC values of >4,096 µg/ml against both bacteria, indicating similarly low efficacy. The PW exhibited the strongest antibacterial activity among the extracts, with an MIC of 2,048 µg/mL against *S. aureus*. However, the MIC value against *E. coli* was still >4,096 µg/mL, indicating limited or no efficacy against this Gram-negative bacterium. For comparison, the reference antibiotics showed significantly lower MIC values, reflecting much stronger antibacterial activity. Vancomycin, a standard antibiotic for Gram-positive bacteria, had a MIC of 0.5 µg/mL against *S. aureus*. Gentamicin, used here as a reference for Gram-negative bacteria, showed MIC values of 0.25 µg/mL against *E. coli*. The antimicrobial activity results reveal that *C. grandis* extracts possess relatively weak inhibitory effects against the tested bacterial strains, especially when compared to conventional antibiotics like vancomycin and gentamicin. Regarding antimicrobial activity, this finding indicates that the antimicrobial effect against *S. aureus* and *E. coli* is not as pronounced as in some of the other studies. The MIC values for our extracts, such as >4,096 µg/mL for LE, LW, and PE, and 2,048-4,096 µg/mL for PW against *S. aureus* and *E. coli*, suggest that these extracts do not exhibit strong antimicrobial activity in comparison to the literature reports. Tao *et al*. (2012) reported that *C. grandis* peel oil had a broad antimicrobial spectrum, but with lower MIC values (4.69 - 37.50 µL) against *S. aureus* and *E. coli* [41]. Saeb *et al*. (2016) found that the essential oils extracted from *C. grandis* leaves exhibited significantly higher antibacterial activity against Gram-negative bacteria, such as *E. coli* (MIC: 150 µg/mL) and *Salmonella typhi* (MIC: 300 µg/mL), compared to their activity against Gram-positive bacteria, including *S. aureus* (MIC: 900 µg/mL) and *Bacillus subtilis* (MIC: 600 µg/mL) [42]. Ezeabara and Dikeh (2019) observed MIC values between 12.5 and 50.0 mg/mL for leaf extracts and much lower MICs for peel and stem bark extracts against *S. aureus* and *E. coli*, while this extract had significantly higher MIC values (>4,096 µg/mL), suggesting that the antimicrobial potency of *C. grandis* extracts (Tubtim Siam) may be relatively lower than that reported by these studies [43]. The lower activity observed in this study may be attributed to a combination of factors, including cultivar differences, extraction methods, concentration of extracts, test organisms, and phytochemical composition.

Anticancer Screening

Both the PE and LE were selected for anticancer analysis based on their high phenolic and flavonoid content and antioxidant activity, which suggest their potential for further biological evaluation against cancer cell lines. The cytotoxicity of PE and LE was evaluated at a maximum concentration of 500 µg/mL MCF-7, A549 and HT-29 cell lines. The results revealed that neither extract exhibited inhibitory effects on the tested cell lines at this concentration. The lack of cytotoxic activity at 500 µg/mL suggests that the ethanoic and water extract of pomelo peels may not directly induce cell death in MCF-7, A549 and HT-29 cancer cells. The lack of cytotoxic activity at 500 µg/mL suggests that the ethanoic and water extract of pomelo peels may not directly induce cell death in MCF-7, A549, and HT-29 cancer cells. Several factors could account for this outcome. While 500 µg/mL is commonly used for initial screening, it might not be high enough to induce cytotoxicity. Higher concentrations or extended exposure times may be required to observe significant effects [44]. Additionally, the phenolic and flavonoid compounds in the extracts may have more subtle impacts, such as inhibiting cell proliferation or inducing cell cycle arrest, rather than causing direct cytotoxicity. These compounds may not possess sufficient potency to induce cell death at the tested concentration [45].

GC-MS analysis

Following a thorough analysis of TPC, TFC, and antioxidant activity across four extracts, the ethanoic extract of pomelo peels (PE) was chosen for further GC-MS analysis. This selection was based on the PE extract's elevated levels of TPC and TFC, coupled with its notable antioxidant activity, suggesting a diverse array of bioactive compounds that merit further chemical investigation. The chemical composition was analyzed based on retention times using a fused silica capillary column. The mass spectral fragmentation patterns of the compounds were compared to reference spectra from the NIST23 and Wiley 23 databases in order to identify them. The identified compounds were classified as bioactive components.

Table 4. GC-MS analysis of PE from *C. grandis*

No.	RT	Compound Name	Formola	Component area	Area%	Classification
1	7.94	Dihydroxyacetone	$C_3H_6O_3$	1754242.879	0.553529431	monosaccharides
2	12.85	*D*-Limonene	$C_{10}H_{16}$	6354289.1	2.005016563	monoterpenes
3	14.32	α-Methyl-α-[4-methyl-3-pentenyl]oxirane methanol	$C_{10}H_{18}O_2$	13436192.67	4.239622784	monoterpenes
4	14.84	*trans*-Linalool oxide	$C_{10}H_{18}O_2$	6795405.415	2.144205307	monoterpenes
5	16.49	3,5-Dihydroxy-6-methyl-2,3-dihydropyran-4-one	$C_6H_8O_4$	2421961.709	0.764219768	heterocyclics
6	17.17	Benzoic acid	$C_7H_6O_2$	2885831.882	0.910588208	carboxylic acids
7	17.41	Ethyl Benzoate	$C_9H_{10}O_2$	2569833.395	0.810878832	esters
8	18.77	4-(1-Aminoethyl)phenol	$C_8H_{11}NO$	2862911.484	0.903355962	aminophenols
9	19.05	5-Hydroxymethylfurfural	$C_6H_6O_3$	12055871.69	3.804079739	heterocyclics
10	21.54	2-Methoxy-4-vinyl-phenol	$C_9H_{10}O_2$	3874993.057	1.222705662	phenols
11	22.23	Limonene glycol	$C_{10}H_{18}O_2$	6259617.362	1.975144079	monoterpenes
12	23.95	Methyleugenol	$C_{11}H_{14}O_2$	3237622.054	1.021591203	phenylpropanoids
13	27.73	Elemicin	$C_{12}H_{16}O_3$	5193497.73	1.638743344	phenylpropanoids
14	31.81	4-(1*E*)-3-hydroxy-1-propenyl-2-methoxyphenol	$C_{10}H_{12}O_3$	1884522.198	0.594637443	phenols
15	33.40	Nootkatone	$C_{15}H_{22}O$	2529607.818	0.798186153	sesquiterpenoids
16	36.31	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	2057090.541	0.649089228	fatty acids
17	36.89	*p*-Methoxytodadiol	$C_{12}H_{18}O_5$	1787538.133	0.564035332	phenylpropanoids
18	41.52	Isoauraptene	$C_{15}H_{16}O_4$	17073414.92	5.387302838	coumarins
19	42.27	Auraptenol	$C_{15}H_{16}O_4$	3269299.67	1.031586679	coumarins
20	43.53	*N*-(4-Methoxyphenethyl)benzamide	$C_{16}H_{17}NO_2$	6606336.182	2.084546872	amides
21	44.67	Meranzin hydrate	$C_{15}H_{18}O_5$	35388363.16	11.16635601	coumarins
22	54.70	Naringenin	$C_{15}H_{12}O_5$	67711510.23	21.36552136	flavonoids

The GC-MS analysis of the ethanoic extract of *C. grandis* peels (PE) revealed 22 distinct compounds, identified based on their retention times, peak areas (%), molecular formulas, match factors (>80.00), and relative abundances (>0.5% of the total composition). These compounds belong to various chemical classes, including flavonoids, coumarins, monoterpenes, and phenylpropanoids, highlighting the extract's diverse bioactive profile. The identified compounds are listed in Table 4, along with their respective chemical formulas, component areas, and classifications. Additionally, the GC-MS chromatogram illustrating these compounds is presented in Figure 1. The flavonoid naringenin was the most abundant compound, constituting 21.37% of the total area. Another major component was meranzin hydrate (11.17%). Among the coumarins, isoauraptene (5.39%) and auraptenol (1.03%) were notable. Monoterpenes were prominently represented, with D-limonene (2.01%) and its oxygenated derivatives, trans-linalool oxide (2.14%) and limonene glycol (1.98%), contributing significantly. Phenolic compounds, such as 4-(1-aminoethyl)phenol (0.90%) and 2-methoxy-4-vinylphenol (1.22%), were also identified. The GC-MS profile further detected fatty acids, including n-hexadecanoic acid (0.65%), and phenylpropanoids, such as methyleugenol (1.02%) and elemicin (1.64%). Notably, 5-hydroxymethylfurfural (3.80%) was identified, a compound known for its diverse health effects, which include both beneficial and adverse impacts [46].

The list of previously reported compounds is accurate based on known literature. For the first time, six compounds were identified in *C. grandis* peels, including 3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one, 4-(1-aminoethyl)phenol, *p*-methoxytodadiol, limonene glycol, 4-(1*E*)-3-hydroxy-1-propenyl-2-methoxyphenol, and *N*-(4-methoxyphenethyl)benzamide. In addition, some compounds has been reported in *C.grandis* such as, dihydroxyacetone, D-limonene, *trans*-linalool oxide, benzoic acid, ethyl benzoate, 5-hydroxymethylfurfural, methyleugenol, 2-methoxy-4-vinyl-phenol, nootkatone, elemicin, n-hexadecanoic acid, meranzin hydrate, isoauraptene, auraptenol, and naringenin [8, 47-48]. This detailed correspondence highlights the robust composition of PE as revealed by GC-MS, aligning it with the results from the phytochemical screening, including flavonoids (naringenin), coumarins (meranzin hydrate, isoauraptene, auraptenol) and terpenoids (D-limonene, α-methyl-α-[4-methyl-3-pentenyl]-oxiranemethanol, trans-linalool oxide, limonene glycol and nootkatone). While 4-(1-amino-ethyl)phenol and N-(4-methoxyphenethyl)benzamide were detected, these compounds are more accurately classified as an aminophenol and an amide, respectively, based on their functional groups and chemical structures, rather than as alkaloids.

By explicitly linking the qualitative phytochemical screening (presence of flavonoids, coumarins, terpenoids) with the quantitative assays (high TPC and TFC) and the detailed GC-MS chemical identification, it becomes evident that the ethanolic peel extract not only harbors a high concentration of bioactive compounds but also a diverse chemical profile. This integrative evidence underscores the critical role of solvent choice, with ethanol proving especially effective in extracting polyphenolic and terpenoid constituents compared to water. Altogether, these findings highlight *C. grandis* peel as a particularly rich source of antioxidant and therapeutic agents, supporting its potential use in functional food development, nutraceuticals, and natural product-based pharmaceuticals.

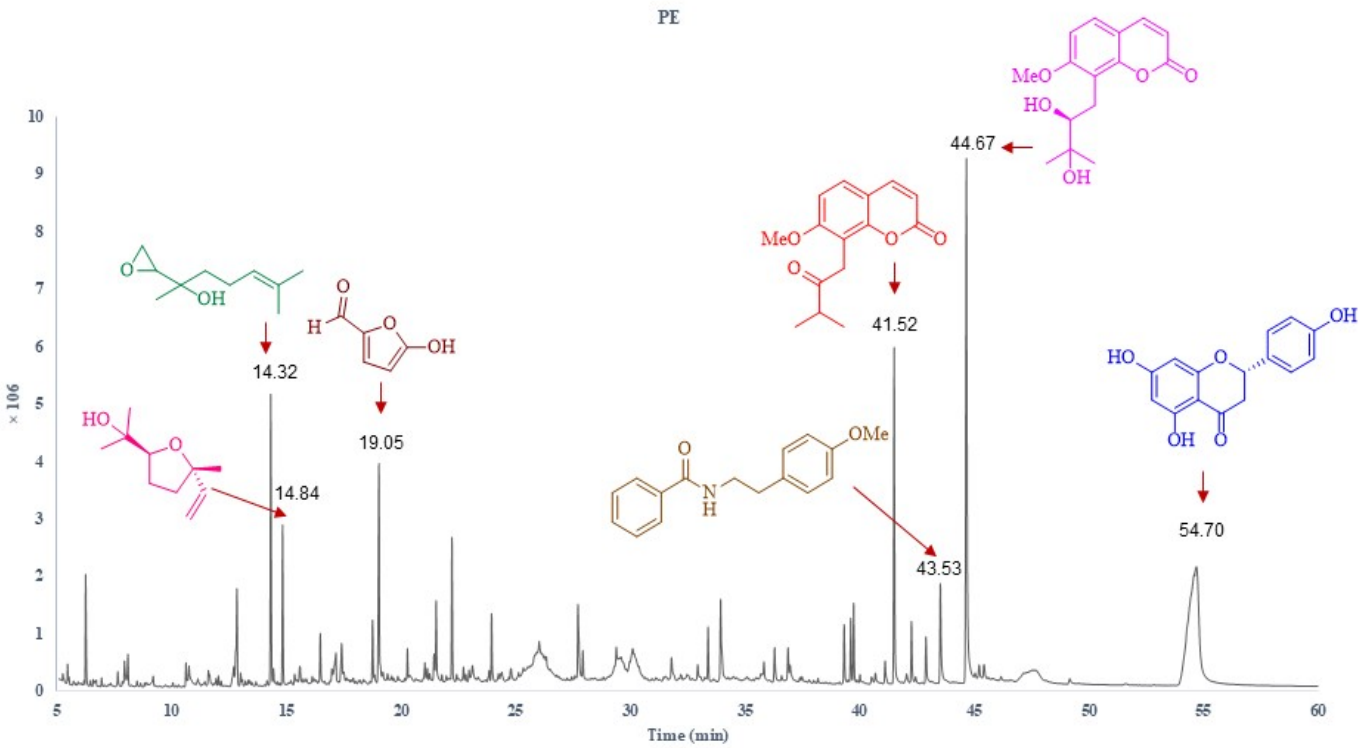

Figure 1. GC-MS analysis of the PE from *C. grandis* of main components

Naringenin is well-known for its potent antioxidant, anticancer, anti-infective, and anti-inflammatory properties. Its high concentration in the ethanoic extract of *C. grandis* peels (PE) highlights the significant health-promoting potential of this extract [49-51]. Furthermore, the presence of naringenin aligns with reports on other *Citrus* species, supporting the use of *C. grandis* peels as a valuable source of bioactive flavonoids. The high concentration of naringenin in the PE extract may also be attributed to the ethanol extraction method, which is particularly effective at solubilizing flavonoids. In addition to naringenin, the identification of meranzin hydrate, a coumarin derivative with reported anticancer and anti-inflammatory properties [52-53], further enhances the value of *C. grandis* as a medicinal plant. The high prevalence of flavonoids and coumarins indicates potential for use in nutraceuticals and functional foods. Moreover, the presence of monoterpenes and phenolic compounds supports the extract's suitability for use in cosmetic and pharmaceutical formulations. Comparatively, the profile aligns with previous studies on *Citrus* species, reinforcing the role of coumarins and flavonoids as key bioactive components. Furthermore, the identification of additional bioactive classes, such as monoterpenes (e.g., D-limonene and trans-linalool oxide) and phenolic compounds (e.g., 2-methoxy-4-vinylphenol), expands the potential applications of the PE extract. D-limonene, a naturally occurring monocyclic monoterpene found in high concentrations in citrus fruits such as lemons, oranges, and grapefruits, is extensively utilized as an additive for flavor and fragrance in diverse products, including perfumes, soaps, foods, and beverages, due to its distinctive and pleasant aroma. This compound is known for its diverse biological activities, including antioxidant, antidiabetic, anti-inflammatory, immunomodulatory and anticancer activities [54-55]. These results demonstrate the potential of *C. grandis* peels as a valuable source of bioactive compounds, supporting their use in the creation of health-enhancing products.

Conclusions

This study provides a comprehensive insight into the bioactive potential of extracts from leaves and peels of *C. grandis* (Tubtim Siam), highlighting their phytochemical richness and biological properties. The ethanol extract of pomelo peel (PE), characterized by a high TPC and TFC, showed the strongest antioxidant activity, consistent with the presence of important bioactive compounds such as naringenin and meranzin hydrate. Remarkably, for the first time, six bioactive compounds were detected in extracts from the peel of *C. grandis* (Tubtim Siam) by GC-MS analysis, extending the known chemical profile of this species. Despite the limited antimicrobial and anticancer activity at the concentrations tested. The high concentration of naringenin, a flavonoid with well-documented health benefits, underlines the suitability of the extracts for use in dietary supplements, functional foods and cosmetic formulations. The study also confirms the ethanol extraction method as a highly effective approach to isolate flavonoid-rich extracts and underlines its potential to optimize extraction strategies in future studies. Furthermore, this approach is an example of the sustainable conversion of agro-industrial waste, such as *C. grandis* peels, into high-value bioactive products that benefit the environment and the economy. Future research should focus on isolating individual compounds for detailed pharmacological testing and investigating potential synergistic interactions between the components in PE. In vivo and clinical studies are essential to validate the therapeutic potential of *C. grandis* peels and optimize their application in health-promoting products.

Conflicts of Interest

The authors have disclosed no conflicts of interest.

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