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PHENOLICS CONTENT, ANTIOXIDANT, ANTIBACTERIAL AND ANTICANCER ACTIVITIES OF POMELO PEELS

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ABSTRACT

Pomelo peel, a byproduct of pomelo consumption, is high in nutrients and useful components such as phenols and flavonoids, yet the majority of it is discarded as waste materials. There is very few studies focus on the phytochemical compounds of pomelo peels, so, the current study investigated the antioxidant, antimicrobial, and anticancer activities of pomelo peels methanolic extract. The results showed that methanol produced the highest phenolics extraction yield (21.48%) and total polyphenols (798.17 mg GAE/g) from pomelo peels powder. HPLC analysis identified 15 phenolic compounds, with salicylic, ellagic, and catechin being the highest (2.19, 1.97 and 1.81 g/kg, respectively). The extract contained seven flavonoid components, with rosmarinic (1219.38mg/100g), hesperidin (424.08 mg/100g) and quercetrin (29.45 mg/100g) being the highest. The methanolic extract had a high antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) but lower than BHT as a standard synthetic antioxidant. The induction period (using the Rancimat instrument) was increased from 8.46 hr for virgin sunflower oil to 10.27 and 12.34 hr for sunflower oil containing 200 and 400 ppm of pomelo peels methanolic extract, respectively, compared with 13.58 hr for sunflower oil containing 200 ppm of BHT. The extract showed significant antimicrobial activity against Gram-positive bacteria and higher activity against HCT116 (human colon cancer) than HEPG2 (human liver cancer). So, pomelo peels could be used as a source of bioactive compounds in functional foods.

Keywords: BHT, DPPH, polyphenols, sunflower oil

INTRODUCTION

Reactive oxygen species (ROS) are extremely unstable and interact quickly with other molecules such as proteins, lipids in membranes, and DNA. Unchecked ROS activity has been associated with diabetes mellitus, inflammation, neurological illnesses, hypertension, gastric ulcers, arthritis, reperfusion, cancer, and other health issues **(Barakat *et al.*, 2022)**. It is commonly recognized that antioxidants may scavenge free radicals and ROS, therefore reducing damage caused by oxidative stress by stopping the radical reaction that results from peroxidation of lipids **(Elsebaie *et al.*, 2022)**. The consumption of foods high in antioxidants is a practical way to regulate the harmful effects of ROS **(Abdelhakam *et al.*, 2019)**. Natural compounds derived from plants have numerous health benefits and play an important role in disease prevention **(Yabalak *et al.*, 2022)**. The rise of drug and treatment resistance in cancer cells and microbes is the primary driver of the increasing demand for innovative antibacterial and anticancer medications. Citrus is a genus of crops in the *Rutaceae* family that are produced in many parts of the world because of their many health and nutritional benefits. Phenol content varies by species, although it is commercially important. However, leaves and seeds have not gotten the same attention as similar fruits **(Ajboory and Al-Douri 2023)**.
Citrus fruits include carotenoid components, flavonoids, polyphenols, and ascorbic acid, making them some of the most potent antioxidants **(Erba *et al.*, 2020)**.The pomelo (*Citrus maxima*) is a citrus fruit that grows widely in tropical and subtropical southern Asia. It contains high antioxidants such as polyphenols, carotenoids, and vitamins, which provide health benefits beyond basic nutrition **(Russo *et al.*, 2021)**.
Pomelo peels, which make up between 30% and 50% (w/w) of the fruit, are a significant by-product of these consumptions. An estimated 2.8 million to 4.7 million tons of pomelo peels were produced worldwide in 2018. Pomelo peel's primary chemical components are carotenoids, flavonoids, and vitamin C. These compounds are closely linked to a wide range of biological activities, including anticough, cardiac stimulant, antioxidant, antiatherogenic, anti-inflammatory, antimicrobial, and anticancer properties **(Liu *et al.*, 2021)**.
Furthermore, earlier research revealed that the pomelo fruit's peel has a greater antioxidant concentration and capacity than its pulp **(Cao *et al.*, 2024)**. Therefore, a variety of valuable goods or components for the culinary, cosmetics, and pharmaceutical sectors may be produced through the intricate use of pomelo peels. The objective of our study was to determine the most suitable solvent for extracting polyphenols from pomelo peels powder as well as to study the antioxidant, antibacterial, and anticancer properties of this extract.

MATERIAL AND METHODS

Materials

In April 2022, commercially mature pomelo fruits (*Citrus maxima*) were gathered from the agricultural faculty's research farm at Kafrelshiekh University in Egypt. The fruits were chosen on the basis of their consistent size, color, and level of commercial ripeness.
Drug cytotoxicity HEPG2 (hepatocellular) and HCT116 (colon) were acquired from the National Institute of Oncology in Cairo, Egypt. Sigma Company for Chemicals and Drugs, St. Louis, MO, USA, provided all of the compounds utilized in this investigation, including methanol, BHT, phenolic acids, and 1,1- diphenyl-2-picrylhydrazyl (DPPH), which were of HPLC grade (99.9% purity).
Microorganisms: Bacterial strains representing Gram-positive (*Bacillus cereus and Staphylococcus aureus) besides Gram-negative (Escherichia coli* and *Pseudomonas aeruginosa*) were obtained from the Microbiology Department, Faculty of Agriculture, Kafrelshikh University, Egypt.

Preparation of samples

To get rid of any dust or sticky materials, pomelo fruits were cleaned under running water. The peels were then manually removed from the fruit section using a sharp knife. The peels were cut into 2x2 cm^2 pieces and minced with a Moulinex blender. After being dried at 50°C, chopped samples were kept in polyethylene bags at 4ºC until further analysis.

Chemical composition of pomelo peels

Moisture, crude protein, ether extract, ash, and crude fiber contents were estimated using the techniques described in the **AOAC (2016)**. The total carbohydrates content was calculated by the difference.

Pomelo peels extracts preparation

To prepare pomelo peel extracts, five grams of pomelo peels were homogenized in 50 mL of each solvent (70% ethanol, 80% methanol, 50% acetone, and 50% distilled water) and kept at room temperature for one day. Then they were filtered using filter paper (Whatman No. 1). Finally, the supernatant was evaporated under vacuum in a rotary evaporator (R300, BUCHI, Switzerland) at 45°C and weighed to determine yield **(Elsebaie *et al.*, 2017)**.

Determination of total phenolic contents

Total phenolic content of pomelo extracts was measured spectrophotometrically using three replicates according to Folin-Ciocalteau method as described by **Essa *et al.* (2025)**. Total phenolic content in the extract was expressed as mg gallic acid equivalent per g dry extract (mg GAE/g dry weight of extract).

Determination of total flavonoid contents

Munhoz *et al.* (2014) described the use of the aluminum chloride test to assess the total flavonoid content. Two milliliters of the same solvent were used to dissolve each extract (0.5 milliliters) at a 500 ppm concentration. The mixture was then supplemented with 0.2 ml of 1M potassium acetate, 0.3 ml of 10% w/v $AlCl_3$, and 2 ml of distilled water. The resulting combination was let to stand for half an hour at room temperature. Sample absorbance at 430 nm was measured. The same volume of distilled water was used in place of aluminum chloride in the blank sample. The standard curve was produced using quercetin as a reference. The amount of flavonoids in the extract was measured in triplicate and represented as mg QE/g dry extract, or mg quercetin equivalent per gram extract.

Identification of phenolic acid and flavonoids

Using high-performance liquid chromatography (HPLC), flavonoids and phenolic acid molecules were identified at the Food Technology Research Institute in Doki, Giza, Egypt. Using HPLC coupled to diode- arrays and electroarrays, flavonoids were identified in accordance with **Mattila *et al.* (2000)**. In accordance with the method described by **Goupy *et al.* (1999)**, phenolic compounds were separated and identified using reverse-phase HPLC/diode array detection.

Pomelo peels methanolic extract antioxidant activity measurement

The methanolic extract's ability to scavenge free radicals was assessed using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) in accordance with **Sousa *et al.* (2008)**. A 0.2 mM DPPH in methanol solution was made, and 2 mL of this solution was mixed with 6 mL of the extract solution at various concentrations (ranging from 50 to 1000 ppm).The mixes were given a good shake and let to stand for half an hour at room temperature. Then, a JENWAY 6100 UV-VIS spectrophotometer was used to detect the absorbance at 517 nm. 200 ppm of butylated hydroxyanisole (BHA) was used as the reference. Higher free radical scavenging activity is shown by lower absorbance values of the reaction mixture. The following formula was used to determine the capacity to scavenge the DPPH radical.

$$\text{Inhibition \%} = \left[\frac{Blank\ absorbance\ -\ Sample\ absorbance}{Blank\ absorbance}\right] \times 100$$

Rancimat test

In comparison to sunflower oil without any additives (control) and sunflower oil with 200 ppm of BHT, pomelo peel methanol extract was added to refined sunflower oil at quantities of 200 and 400 ppm. According to **Dıraman and Baydır (2017)**, the Rancimat technique was used to assess the oxidative stability (induction duration, protection factor, antioxidant activity, and rising index) of earlier sunflower oil samples using 679 Rancimat (Metrohm, Herisan, Switzerland). The reaction vessel cylinder was filled with samples, and during the experiment, the air supply was maintained at 20 milliliters per minute and 100 degrees Celsius plus or minus 2.0. While protection factor (PF), antioxidant activities (AA), and increasing index (II) were computed using the following formulas, the induction period (IP) was automatically recorded during determination by hours:

$$PF = \frac{IP\ \textbf{of sample containing extract}}{IP\ \textbf{of control sample without antioxidants}}$$

$$AA = \frac{IP\ \textbf{of sample containing extract}\ -\ IP\ \textbf{of control sample without antioxidants}}{IP\ \textbf{of sample containing BHT}\ -IP\ \textbf{of control sample without antioxidants}}$$

$$II = \frac{IP\ \textbf{of sample containing extract}}{IP\ \textbf{of control sample without antioxidants}} \times 100$$

Antimicrobial activity measurement

Antibacterial activity was evaluated using the conventional agar disc diffusion technique **(Elsebaie and Essa 2022)**. Filter paper discs were impregnated with 10 µl of pomelo peel methanolic extract and allowed to dry. Sterile forceps were used to carefully apply the loaded discs to the surface of the agar plates that had been seeded. Five duplicates of the experiment were conducted, and following a 24-hour incubation period at 37°C, the diameters of the zones of inhibition were determined on a millimeter scale. Broad-spectrum antibiotics (ciprofloxacin at 100 mg/ml) were used as a positive control to compare the outcomes. Additionally, as a negative control, a disc was made using 95% methanol rather than pomelo peel extract.

Anticancer Activity

The anticancer activity of pomelo peel methanolic extract was determined using the Neutral Red Uptake (NRU) technique, as described by **Essa and Elsebaie (2018)**. This method relies on the ability of viable cells (HCT116 human colon carcinoma and HEPG2 human hepatocellular carcinoma) to incorporate and bind the supravital dye neutral red in lysosomes. Cell lines from colon and hepatocellular carcinoma were seeded in 96-well plates and treated with pomelo peel powder methanolic extract at 0, 5, 12.5, 25, and 50 µg /mL. Plates were incubated with neutral red media for 3 hours at 37ºC. Cells were washed, dye removed, and absorbance measured at 540nm.

Statistical Analysis

The data were statistically assessed using SPSS's analysis of variances software and the statistical significance was examined using one-way ANOVA at $p < 0.05$.

RESULTS AND DISCUSSION

Chemical composition of pomelo peels

Many variables influence the chemical composition of pomelo peels, including variations in the organic components of the soil, varietals, fertilizers used, and meteorological and environmental circumstances. The findings in Table (1) show that pomelo peels provide a rich source of protein, crude fiber, and total carbs, all of which are significant nutritionally. The findings found that the moisture, crude protein, ether extract, ash, crude fiber, and total carbohydrate contents were 8.43, 8.14, 3.82, 5.18, 34.62, and 74.43%, respectively. These results were in harmony with those of **Van Hung *et al.* (2020a)** and **Yin *et al.* (2023)**.

Table 1 Gross chemical composition of pomelo peels powder (on dry weight basis)

Components	Pomelo peels powder
Moisture (%)	8.43±0.76
Crude protein (%)	8.14±0.65
Ether extract (%)	3.82±0.42
Ash (%)	5.18±0.78
Crude fiber (%)	34.62±1.29
Total carbohydrate (%)*	74.43±1.54
Total phenol content (mg GAE/g DW)	5.53±0.28
Total Flavonoids content (mg QE/g DW)	2.86±0.16

*Total carbohydrates were calculated by difference. Values are means of three replicates ± standard deviation (SD).

Phenolic compounds are macromolecules made up of an aromatic chain and a hydroxyl group that are thought to be a primary source of antibacterial action **(Barnes and Karatzas 2020)**. Several variables influence the phenolic content of pomelo peel, including geographical origin, environmental, physiological, and nutritional variances **(Masniyom *et al.*, 2012)**. The Folin-Ciocalteu test is one of the earliest procedures for determining total phenol concentrations. In this study, it should be mentioned that the total polyphenol content in pomelo peel powder, reported as mg gallic acid equivalent/g, was 5.53 mg GAE/g. Our findings were consistent with those published by **Yin *et al.* (2023)**, who noted that the quantities of total phenols in six distinct pommel types grown in China ranged from 5.43 to 11.97 mg/100g. Furthermore, data reported in the same table revealed that the overall flavonoid concentration in pomelo peel powder, represented as mg quercetin equivalent/g, was 2.86 mg QE/g. These findings were lower than those who recorded that the total flavonoids content in pomelo peels powder was 1.80 mg quercetin equivalent/g.

The Effect of using various solvents on polyphenols extraction from pomelo peels powder

Total extraction yield and phenol content of pomelo peels are tabulated in Table 2. The results disclosed that using water gave the highest value for extraction phenolic yield, amounting to 24.80 %, followed by methanol (80%) with 21.48 %.

Table 2 Extraction of using various solvents during polyphenols extraction from pomelo peels powder on extraction yield and total polyphenols content

Solvent	Extraction yield (%)	Total polyphenols (mg GAE/g)
Methanol 80%	21.48±0.98^{c}	798.17±5.20^{a}
Ethanol 70%	18.72±1.09^{d}	580.36±3.66^{b}
Aceton(50%)	26.12±0.86^{a}	472.51±3.98^{c}
Water	24.80±0.59^{b}	297.11±2.54^{d}

Values are means of three replicates ± standard deviation (SD). In a column, means with the same small superscript letters are not significantly different at $p < 0.05$

In addition, the results refer to methanol as the best solvent for extracting polyphenolic components from pomelo peels powder. High amounts of polyphenolic compounds were given with methanol (798.17 mg GAE/g), in comparison with others. These results are in accordance with those achieved by **Kumar and Goel (2019)**.

Identification of phenolic acids content of pomelo peels methanolic extract

The HPLC technique was used to fractionate and identify the phenolic components extracted from the test sample. The results were tabulated in Table (3).
The results revealed that pomelo peels powder methanolic extract has 15 phenolic components. Salicylic was the highest compound of polyphenolic compounds found in methanolic extract (2.19 g/kg) followed by ellagic (1.97 g/kg), where cinnamic (0.03 g/kg) and caffeine (0.03 g/kg) were the lowest ones. The obtained results were in accordance with **Lin *et al.* (2021)**, who stated that pomelo peels methanol extract contains different phenolic compounds such as caffeic, gallic, pyrogallol, benzoic and chlorogenic where the most present phenolic compounds were salicylic acid and ellagic acid.

Table 3 Phenolic compounds (g/kg) in pomelo peels methanolic extract

Phenolic compound	Content (g/kg)
Chlorogenic acid	0.22±0.01
Catechin	1.81±0.02
Caffeine	0.03±0.00
Cinnamic acid	0.03±0.00
Coumarin	0.08±0.00
Caffeic acid	0.09±0.00
Chrysin	0.18±0.01
Vanillic acid	0.10±0.02
Ellagic acid	1.97±0.02
Salycilic acid	2.19±0.05
Protocatchuic acid	0.18±0.01
Catechol	0.34±0.02
Benzoic acid	0.58±0.03
Gallic acid	0.04±0.00
Syringic acid	0.10±0.01

Values are means of three replicates ± standard deviation (SD).

Identification of flavonoid compounds of pomelo peels methanolic extract

HPLC procedure was utilized for fractionating and identifying the flavonoid components extracted from the tested sample. The results were tabulated in Table 4. The data obtained revealed that pomelo peels methanolic extract has seven flavonoid components. Rosmarinic was the highest compound of flavonoid compounds found in the methanolic pomelo peels extract (1219.38 mg/100 g) followed by hesperidin (424.08 mg/100 g) then quercetin (29.45 mg/100g), where naringenin (1.16 mg/100 g), apigenin (2.84 mg/100 g) and kaempferol (6.94 mg/100 g) were the lowest ones. These results were in the same line with those of **Tran *et al.* (2021)**.

Table 4 Flavonoid compounds (mg/1oo g extract) in pomelo peels methanolic extract

Flavonoid compounds	Content (mg/100 g)
Hesperidin	424.08±2.19
Quercetrin	52.35±0.83
Naringenin	1.16±0.06
Kampferol	6.94±0.10
Apigenin	2.84±0.23
Rosmarinic	1219.38±2.97
Querctin	29.45±0.92

Values are means of three replicates ± standard deviation (SD).

Antioxidant activity of pomelo peels methanolic extract

In this assay, antioxidant activity was also represented as IC_{50} (antioxidant activity expressed as an inhibition percentage equivalent to a 50% reduction in DPPH absorbance). The 1,1-diphenyl-2-picryl-hydrazil (DPPH) scavenging activity has been widely employed to identify antiradical activity of diverse samples, since it is sensitive to lower amounts of active components from natural sources.
The stable radical, DPPH has a maximum absorbance at a 517nm wavelength and could readily undergo scavenging by antioxidants. Higher free radical scavenging activity of samples is indicated by lower absorbance. BHT exhibited a significant scavenging activity at $P<0.05$ when compared to pomelo peel powder methanolic extract (Figure 1). In fact, the DPPH scavenging activity of pomelo peel powder methanolic extract substantially elevated as the concentration increased from 50 to 1000 ppm (Figure 1).
The percentages of DPPH inhibition for pomelo peel powder methanolic extract were 37.20, 48.54, 56.12, 63.50, 74.26, and 83.43% for the following methanolic extract concentrations: 50, 100, 200, 500, 800, and 1000 ppm, respectively.

It is also apparent from the same figure that a 1000 ppm concentration of pomelo peel methanolic extract had relatively high antioxidant activity, close to that of BHT (89.72) as a synthetic antioxidant. Also, data in the same table indicated that IC_{50} value for pomelo peel methanolic extract was 119.26 ppm.

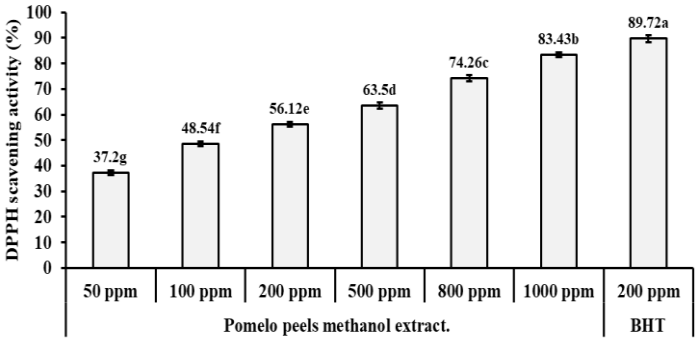

Figure 1 DPPH radical scavenging activity (%) of pomelo peels methanol extract. Values are means of three replicates ± standard deviation (SD). Means with the same small superscript letters are not significantly different at $p < 0.05$

Oxidative stability of sunflower oil as affected by addition of pomelo peels methanol extract

The antioxidant efficiency of pomelo peels phenolic extract was evaluated. Refined blended oil, free of antioxidant, was used as the material for oxidation studies.
Refined oil samples containing 200 and 400 ppm of pomelo peels methanolic extract. Synthetic antioxidant (BHT) was used as a positive control at their legal limit of 200 ppm. Control samples without antioxidant were also placed under identical conditions. All oil samples are subjected to accelerated oxidation at Rancimat air flow 20 L/hr at 100ºC. All oil samples of each treatment were withdrawn every hour to assess the antioxidant activity of pomelo peels methanolic extract.
Data in Table 5 indicated that the sunflower oil oxidative stability parameters (induction period, protection factor, antioxidant activities, and increasing index) were increased with the addition of pomelo peels methanolic extract compared with the control. Also, data in the same table revealed that there was a significant increase in the sunflower oil oxidative stability parameters when the pomelo peels extract was increased from 200 ppm to 400 ppm.
It was clear that the addition of antioxidants extracted from pomelo peels methanolic extract increased the stability of sunflower oil at all level (200, 400ppm) compared with the control. The induction period of sunflower oil increased from 8.46 hr for control to 10.27 and 12.34 hrs. Meanwhile, it was increased to 13.58 hrs by the addition of BHT at 200 ppm (Table 5).
The antioxidant activity was calculated and also presented in the same table. Hence, it could be noticed that with the increase in the pomelo peels methanol extract added to sunflower oil from 200 ppm to 400 ppm the antioxidant activity value was a significant increase from 0.35 to 0.76, but it was still lower than that obtained by sunflower oil containing 200 ppm BHT. In general addition of antioxidants that extracted from pomelo peels methanol extract to sunflower oil led to increasing in induction periods as a result of increasing of antioxidant activities.
According to the findings in Table (5), increasing the concentration of pomelo peel extract in sunflower oil from 200ppm to 400ppm resulted in a substantial rise in both the protection factor and the rising index percentages. When the concentration of pomelo peel extract in sunflower oil rose from 200ppm to 400ppm, the protection factor climbed from 1.21 to 1.46, while the rising index percentage increased from 121.39% to 145.86%.
In general, BHT at 200 ppm had the greatest values for induction period, protection factor, antioxidant activities, and rising index when compared to pomelo peel methanol extract at all doses. The results showed that the methanol extract of pomelo peels has antioxidant properties that helped to slow down oil oxidation. The obtained results were in line with those obtained by **Helal *et al.* (2020)**, who reported that synthetic antioxidants remained the most effective and gave the highest induction period compared to natural extracts, because synthetic antioxidants are pure constituents, whereas natural extracts were in complex mixtures with active compounds being present at low concentrations but considered safer for human consumption than synthetic antioxidants that have potential health.

Table 5 Stability index of virgin sunflower oil with pomelo peels methanolic extract using Rancimat apparatus at 100°C.

Oil sample	Induction period (hr)	Antioxidant activities	Protection factor	Increasing index (%)
sunflower oil	8.46[d]	0	1.00[d]	100[d]
sunflower oil +BHT200ppm	13.58[a]	1.00[a]	1.61[a]	160.52[a]
Sunflower oil+200ppm pomelo peels extract	10.27[c]	0.35[c]	1.21[c]	121.39[c]
Sunflower oil+400ppm pomelo peels extract	12.34[b]	0.76[b]	1.46[b]	145.86[b]

Values are means of three replicates ± standard deviation (SD). In a column, means with the same small superscript letters are not significantly different at $p < 0.05$.

Antimcrobial activity of pomelo peels methanolic extract

The antibacterial properties of pomelo peels methanolic extract against gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) were evaluated and compared with ciprofloxacin (5 µg/ml). The sizes of inhibition zones were used to determine the antibacterial efficacy of the extracts. The obtained results are given in Table 6.

Data given in Table 6 showed that pomelo peels methanolic extract showed lower antibacterial activity compared with ciprofloxacin. In addition, the pomelo peels methanolic extract gave the highest wide inhibition zones (12.9 mm) against *Staphylococcus aureus*, followed by inhibition zones (12.2mm) against *Bacillus cereus*, inhibition zones (11.1mm) against *Escherichia coli* and inhibition zones (10.6mm) against *Pseudomonas aeruginosa*. **Van Hung *et al.*(2020b)** found that, the variation in the inhibitor effect may be due to the presence of biomolecules in the extract. Moreover, **Mastuki *et al.* (2024)** reported that phenolic acids content of plant extracts act on the membrane or cell wall of the microorganisms, causing structural and functional damage.

According to the same table, the inhibitory effect of pomelo peel methanolic extract on Gram-positive bacteria was substantially larger than that on Gram-negative bacteria. These findings are consistent with those of **Al-Saman *et al.* (2019)**, who discovered that plant extracts show significant antibacterial activity against microbes. Thus, they can be utilized to treat infections caused by resistant microorganisms.

Furthermore, **Shakya (2019)** discovered that the maximum resistance in Gram negative bacteria might be attributed to the existence of an outer membrane wrapping the cell wall, which inhibits the passage of hydrophilic substances via its lipopolysaccharide coating. The absence of this brazier in Gram-positive bacteria allows hydrophobic compounds to come into direct contact with the phospholipid bilayer of the cell membrane, increasing permeability and leakage of intracellular compounds as well as reducing the bacterial enzyme system. However, most investigations have found that plant extracts are more effective against Gram-positive bacteria than Gram-negative ones. The findings of **Asghar *et al.* (2022)** are consistent with ours.

Table 6 Diameters of inhibition zones (mm) resulted from antimicrobial effects of pomelo peels methanolic extract toward some microorganisms

Examined microorganisms	Diameter of inhibition zones (mm)	
	Pomelo peels methanolic extract	Ciprofloxacin
Gram-negative bacteria		
Escherichia coli	11.1±0.1^{Bc}	35.4±0.2^{Aa}
Pseudomonas aeruginosa	10.6±0.1^{Bd}	33.1±0.4^{Ab}
Gram-positive bacteria		
Bacillus cereus	12.2±0.2^{Bb}	30.8±0.3^{Ad}
Staphylococcus aureus	12.9±0.1^{Ba}	31.4±0.2^{Ac}

Values are means of three replicates ± standard deviation (SD).
In a row, means with the same capital superscript letters are not significantly different at $p < 0.05$.
In a column, means with the same small superscript letters are not significantly different at $p < 0.05$.

Anticancer activity of pomelo peels methanolic extrac

Pomelo Peels methanolic extract was evaluated for anticancer activity *in vitro* disease orient antitumer screening using neutral red up take (NRU)assay including two human tumor cell lines representing different cancer types, where HCT116 (Human colon cancer) and HEPG2 (Human liver cancer) cancer cell lines.

Table 7 Anticancer activity of pomelo peels methanolic extract

Concentrations (µg/ml)	Dead cells %	
	HCT116 (Human colon cancer)	HEPG2 (Human liver cancer)
0.0	---	---
5.0	34.70±0.98^{Ad}	21.45±0.94^{Bd}
12.5	42.64±1.08^{Ac}	32.86±1.25^{Bc}
25.0	59.77±1.03^{Ab}	56.13±0.88^{Bb}
50.0	79.18±1.15^{Aa}	74.24±0.98^{Ba}
IC_{50}	21.09µg/ml^{B}	23.28µg/ml^{A}

Values are means of three replicates ± standard deviation (SD).
In a row, means with the same capital superscript letters are not significantly different at $p < 0.05$.
In a column, means with the same small superscript letters are not significantly different at $p < 0.05$.

As indicated in Table 7, the methanolic extract of pomelo peel powder demonstrated a variety of cytotoxic properties against the two lines of human cancer cells tested. In general, the decrease in tumors in cell lines was dose-dependent. However, increasing the concentration of methanolic extract resulted in a greater number of dead cells. The maximum HCT116 dead cell percentage was achieved with 50 µg /ml at 79.18%, while the lowest dead cell percentage was reported with 5.0 µg /ml at 34.70%. On the other hand, the greatest HEPG2 dead cell percentage was observed when using 50 µg /ml with 74.24%, while the lowest dead cell percentage was obtained when using 5.0 µg /ml with 21.45%. These results are consistent with those of **Wu *et al.* (2022)**.

Overall, polyphenols are appealing chemicals for cancer treatment because they may exhibit anticancer effects through a variety of methods, including cancer cell elimination via signaling system change, cell cycle inhibition, and apoptosis induction. Polyphenols also affect the activity of enzymes involved in tumor cell growth. Recent research has linked natural polyphenols to anti-cancer activity via a variety of features, including antiangiogenic, antimetastasis, DNA interaction, and others **(Rathod *et al.*, 2023)**.

CONCLUSION

Pomelo peels are a rich source of protein, crude fiber, and total carbohydrates. Their total polyphenol content is 5.53 mg GAE/g, with a flavonoid concentration of 2.86 mg QE/g. The extraction yield and phenol content are 24.80% using water and methanol, with methanol being the best solvent for extracting polyphenolic components. The methanolic extract contains 15 phenolic components, with salycilic being the highest compound. The antioxidant activity of the extract is significantly elevated as the concentration increases. Also, the study found that increasing the concentration of pomelo peels extract in sunflower oil from 200ppm to 400ppm significantly increased the protection factor and rising index percentages. The methanol extract of pomelo peels showed antioxidant properties, slowing down oil oxidation.The methanolic extract also shows antibacterial properties against gram-negative and gram-positive bacteria, with the highest wide inhibition zones against Staphylococcus aureus. It also demonstrated cytotoxic properties against two human cancer cell lines, HCT116 and HEPG2. Polyphenols are attractive for cancer treatment due to their potential anticancer effects through various methods, including cancer cell elimination, cell cycle inhibition, and apoptosis induction. Generally, pomelo peels powder methanolic extract has a great activity as an antioxidant, antibacterial, and anticancer agent that may be utilized to make functional meals that give humans with the nutrients they require while also protecting them from certain diseases.

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