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Antitumor and cytotoxic activity of watery *Citrus maxima* L. peels extract

Fadia Hameed MOHAMMED *[1], Israa Abd ALRYAHI [2], Ghofran Maher Abed AL- HAMEED [3], Yazi Abdullah JASSIM [1]

[1] Department of Biology, College of Science, University of Babylon, Hillah, Iraq.
[2] Ministry of Education, Babylon Education Directorate, Iraq.
[3] College of Health and Medical Technologies, Al-Zahraa University for Women, Karbala, Iraq.
*E-mail: sci.fadyah.hammed@uobabylon.edu.iq

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ABSTRACT: It is now emphasized that phytotherapy could treat many cancer types and provide cancer cells with a targeted, efficient treatment. Additionally, it is employed to address the cancer cells' resistance to chemotherapy. The current research aimed to assess the impact of pomelo peel extract on human brain cancer A172 and human pancreas cancer. *Citrus maxima* L. pomelo peels were extracted using water, while their phytochemical composition was examined using GC-MS and preliminary phytochemical analysis. Human brain cancer A172 and pancreatic cancer are cytotoxic to pomelo peel extract. Capan-2 cell lines were investigated using the MTT test. A multi-parameter cytotoxic assay was carried out to find the extract action on valid cell count, total nuclear intensity, mitochondrial membrane potential, cell membrane permeability, and cytochrome C release utilizing the HCS test. The results demonstrated the extract's richness in several phytochemical constituent categories, such as phenolics, terpenoids, and alkaloids. MTT assay results indicated that IC50 (994.826 µg mL^{-1}) had no effects on the viability of normal mesenchymal cells, whereas A172 cells (IC50 (265.396 µg mL^{-1}) and Capan-2 cells (IC50 (192.247 µg mL^{-1}) exhibited considerable cytotoxicity. Subsequent analysis of the HCS results revealed notable variations in every parameter examined at concentration levels of 200 and 400 µg mL^{-1}. The extract is a strong antioxidant, rich in many therapeutic phytochemical compounds, and highly toxic to A172 and Capan-2 cells, but Capan-2 cells are more sensitive to the extract. Pomelo peel extract didn't appear toxic to normal mesenchymal cells (HdFn).
Keywords: pomelo peels; cytotoxicity; Brain Cancer A172; Pancreas Cancer Capan-2.

Atividade antitumoral e citotóxica do extrato aquoso da casca de *Citrus maxima* L.

RESUMO: A fitoterapia pode ser usada para tratar muitos tipos de câncer pois pode fornecer às células cancerígenas um tratamento direcionado e eficiente. Além disso, é empregado para tratar a resistência das células cancerígenas à quimioterapia. O objetivo da pesquisa atual foi avaliar o impacto do extrato de cascas de pomelo no câncer cerebral humano A172 e no câncer de pâncreas humano. As cascas de Citrus maxima L. pomelo foram extraídas com água, enquanto sua composição fitoquímica foi examinada por GC-MS, bem como análise fitoquímica preliminar. O câncer cerebral humano A172 e o câncer de pâncreas são citotóxicos para o extrato de cascas de pomelo. Linhas celulares Capan-2 foram investigadas com o uso do teste MTT. Para encontrar a ação do extrato na contagem de células válidas, intensidade nuclear total, potencial de membrana mitocondrial, permeabilidade da membrana celular e liberação de citocromo C utilizando o teste HCS, foi realizado um ensaio citotóxico multiparâmetro. A riqueza do extrato em diversas categorias de constituintes fitoquímicos, como fenólicos, terpenóides e alcalóides, foi demonstrada pelos resultados. Os resultados do ensaio MTT indicaram que IC50 (994,826 µg mL^{-1}) não teve efeitos na viabilidade de células mesenquimais normais, enquanto células A172 (IC50 (265,396 µg mL^{-1}) e células Capan-2 (IC50 (192,247 µg mL^{-1}) exibiram considerável citotoxicidade. A análise subsequente dos resultados do HCS revelou variações notáveis em todos os parâmetros examinados a níveis de concentração de 200 e 400 µg mL^{-1}. O extrato tem sido um forte antioxidante, rico em muitos compostos fitoquímicos terapêuticos e apresenta alta toxicidade para células A172 e células Capan -2, mas as células Capan-2 foram mais sensíveis ao extrato. O extrato de cascas de pomelo não apresentou toxicidade para as células mesenquimais normais (HdFn.)
Palavras-chave: cascas de pomelo; citotoxicidade; câncer cerebral A172; câncer de pâncreas Capan-2.

1. INTRODUCTION

Pomelo (*Citrus maxima* L.) grows in many eastern countries, such as China and Japan, and because of its many benefits, these people consider pomelo a great wealth. There are several types of pomelos, white, red, or pink, and all types are distinguished by minerals and vitamins, which give them many benefits (SAPKOTA et al., 2022).

Pomelo peels protect and reduce the risk of urinary tract infections. Pomelo contains vitamin C (Vitamin C), which

causes it to raise the acidity level in the urine, creating an unsuitable environment for bacteria to grow, thus reducing your risk of urinary tract infection (SINGH, 2017).

Pomelo peels contain high potassium levels, which are important for regulating inflammation (SINGH, 2016). One of the health benefits of pomelo is that eating a full serving of it daily prevents free radicals from attacking the body and causing inflammation (KUNDUSEN et al.,2011). Pomelo peels can also stimulate antibodies and immune cells to fight various bacteria (VIJAYALAKSHMI; RADHA, 2016).

Phenols and flavonoids, antioxidants that lower the risk of pancreatic and breast cancer, are abundant in pomelo peels (PANHEKAR; SAWANT, 2017). Its high dietary fiber content lowers the incidence of colon cancer, and eating pomelo stops cancer cells from spreading throughout the body (ATHIRA, 2017). The majority of cancer patients exhibit an imbalance in the generation of antioxidants as well as free radicals, which is noticeable in cancer patients even after undergoing a variety of treatments, including hormone therapy, immunotherapy, chemotherapy, radiation therapy, and resection surgery (TENIENTE et al., 2023). However, not every patient is a candidate for recovery or a response, and it could even be harmful to the patient (SMAOUI et al., 2019). Pomelo peels are used for treating cancer because they shield the body from the damaging effects of free radicals. Thus, our research aimed to gather pomelo peel, isolate its active ingredients, and examine the anti-cancer potential of these ingredients with the use of many cancer cell lines.

2. MATERIALS AND METHODS

2.1. Preparation of pomelo raw powder (*Citrus maxima* L.) peels

Ripe Pomelo (*Citrus maxima* L.) peels were used for extraction. Pomelo was obtained from the local market and then identified by Dr. Shaemaa Muhi (taxonomist from the Department of Biology at the University of Babylon College of Science). The Pomelo peels were cut into small parts for about 2 x 2 cm. These parts were dried in an oven under 45 °C. Then, the dried pieces of Pomelo peels were milled by an electric miller to make a fine powder. This powder is stored in a sterile dark glass container until it is used for extraction (ASIF et al., 2019).

2.2. Preparation of watery extract of pomelo peels

The raw powder of pomelo (*Citrus maxima* L.) peels was extracted with water. Firstly, the mixture was prepared by mixing one gram of raw powder of pomelo peels with 10 mL of water. Secondly, the mixture was shaken at a high speed for 1 hour. After that, the shaken mixture was put in a water path under 40 °C for 2 hours. Then, filter paper was used to filter the mixture. The filtrated liquid was dried to get a solid texture in an oven under 45 °C. Then, the solid extract was milled to fine powder. Ultimately, the last powder was sterilized by UV light for 20 minutes and then saved in a sterile, dark, and closed cup for use (EKPENYONG et al., 2012).

2.3. Preliminary phytochemical analysis tests

A preliminary phytochemical screening has been conducted on the warry extract of Pomelo peels to identify the main chemical groups. Unless specified differently in each test, a 10 % (w/v) solution of the extract in 50 % methanol was employed in each test (EKPENYONG et al., 2012).

2.4. Screening for alkaloids

Screening about the presence of alkaloid compounds was performed by three tests:

2.4.1. Dragendroffs test

The reagent was prepared by applying the following steps:

1- Distilled water (10 mL) was used for dissolving 0.5 g of bismuth nitrate.
2- Ten mL of Absolute HCl has been added, and then the mixture is stirred.
3- Ten milliliters of distilled water were added to another beaker containing four grams of potassium iodide, and the mixture was stirred until the KI was completely dissolved.
4- The two solutions were mixed to prepare Dragendroffs reagent

Ten milliliters of extract were treated with a few drops of the Dragendroff reagent to conduct the test. Alkaloid compounds are indicated by orange precipitates (QAISAR et al., 2009).

2.4.2. Mayer test

Mayer reagent was prepared freshly by dissolving a mix of potassium iodide (5.0 g) and mercuric chloride (1.36 g) in (100 mL) of distilled water. Then, ten ml of extract was treated with a few drops of Mayer's reagent to perform the test. White precipitates indicate the presence of alkaloids (QAISAR et al., 2009).

2.4.3. Wagner's test

To create Wagner's reagent, dissolve 2.0 grams of iodine and 0.6 grams of KI in 100 mL of distilled water. The test was run by treating one milliliter of an extract with a few drops of Wagner's reagent. A reddish-brown precipitate will occur if alkaloids are present in the solution (QAISAR et al., 2009).

2.5. Screening for phenols

The reagent for phenols (a 1.0 % solution of ferric chloride) is prepared by dissolving 1.0 g of ferric chloride in a small amount of distilled water and then adding more distilled water to make the solution reach 100 mL. After adding three milliliters of plant extract to two milliliters of 1% ferric chloride to conduct the testing, the presence of phenols was confirmed by the emergence of a bluish-green color (EKPENYONG et al., 2012).

2.6. Screening for flavonoids

The flavonoid reagent (1.0 % lead acetate solution) was prepared by dissolving 1.0 g of lead acetate in a small amount of distilled water and then adding more distilled water to make the solution reach a volume of 100 mL. The test was then run using 5.0 mL of the extract and 1.0 mL of lead acetate reagent. The presence of flavonoids is indicated by the formation of a flocculent white precipitate (BANDARNAYAKE, 2002).

2.7. Screening for tannins

By combining 10 mL of absolute ferric chloride alcohol with 90 mL of distilled water, the tannin reagent (10 % ferric chloride alcoholic) has been created. The test was run by adding two to 3.0 mL of the extract to a 10% alcoholic ferric

chloride solution. Tannins are indicated by the solution's dark blue or greenish-gray coloring (QAISAR et al., 2009).

2.8. Screening for coumarins

Sodium hydroxide solution (1.0 M) was the coumarin reagent. Prepared by dissolving 4.0 g of NaOH in a small amount of pure water and adding more distilled water to make the solution reach 100 milliliters. The test was run by adding five milliliters of plant extract to the test tube, covering it with filter paper soaked in one milliliter of NaOH, boiling the tube for five minutes, and then exposing it to a UV light source. According to Patra et al. (2009), the presence of green suggests the presence of coumarins.

2.9. Screening for glycosides

To screen the presence of glycoside compounds, Benedict reagent was prepared by mixing the two following solutions and then completing the mixture with distilled water to one liter:

A- Sodium citrate (173 g) was dissolved, and (100 g) of sodium carbonate in (600 mL) of distilled water with gentle heating and shaking.

B- Copper sulfate (17.3 g) was dissolved in (150 mL) of distilled water.

After that, the test was run by mixing one milliliter of plant extract with 500 µL of Benedict reagent and boiling the mixture for ten minutes. According to PATRA et al. (2009), the presence of pink or red color indicated a favorable outcome.

2.10. Screening for saponin

A foam test was run to determine whether saponin components were present. One milliliter of the extract was diluted with twenty milliliters of distilled water. The solution was violently agitated inside a graduated cylinder for roughly fifteen minutes. A one-centimeter layer of foam formed, verifying the presence of saponins in the sample (BANDARNAYAKE, 2002).

2.11. Screening for resins

The procedure for screening for the presence of resin compounds followed instructions (HAFIDH et al., 2018), which called for mixing 5 g of powdered plant extract with 50 mL of ethanol, heating the mixture to a boil for two minutes, filtering the mixture after it cooled, and then adding 10 mL of distilled water with 4% HCl to the filtered mix. Turbidity's appearance indicates the presence of resin.

2.12. Screening for carbohydrates

To screen the presence of carbohydrate compounds, two tests were performed:

2.12.1. Fehling test

When employing the Fehling reagent, equal quantities of the following solutions were mixed.

1- Copper sulfate solution: $CuSO_4.4H_2O$ (34.66 g) should be dissolved in water and then diluted to 500 mL.

2- Alkaline tartrate solution: 500 mL of water is created by diluting 173 g of potassium sodium tartrate and 50 g of NaOH in water.

A small amount (3 mL) of the extract was diluted in 4 mL of distilled water and filtered to conduct the test. Fehling's solution was applied to the filtrate. Carbohydrates are indicated by a brown color development (EKPENYONG et al., 2012).

2.12.2. Molisches test

Molisch's reagent was made by dissolving 15 g of 1-naphthol in 100 mL of either chloroform or alcohol. Subsequently, 4 mL of distilled water has been mixed with 3 mL of the extract and filtered. Molisch's reagent was applied to the filtrate. Carbohydrates are present when a reddish-brown ring forms (QAISAR et al., 2009).

2.13. Screening for steroids/terpenoids (Liebermann-Burchard test)

One milliliter of the extract, one milliliter of chloroform, two to 3 mL of acetic anhydride, and two to three drops of concentrated H_2SO_4 have been added. The solution's dark green color indicates the presence of steroids, and its dark pink or red color indicates the presence of terpenoids (BANDARNAYAKE, 2002).

2.14. Screening for essential oil

The plant extract was applied to the filter paper, which was then exposed to a UV light source for five minutes before being seen beneath the light. According to Qaisar et al. (2009), the presence of essential oils has been identified by its bright pink color.

2.15. GC-MS analysis for chemical components of pomelo peels watery extract

Agilent, USA instrument 30 mm x 0.25 mm ID x 0.25 µm of capillary column has been utilized for GC-MS study. The injection temperature has been kept at 250 °C, the ion source temperature has been kept at 230 °C, and the helium flow rate has been kept at 1.5mL min^{-1}. The injection has been done with a volume of 1 µL and in the spitless mode. The instrument's temperature was initially set to 70 and held for three minutes. After this time, the oven's temperature was raised to 300 °C and held there for nine minutes, increasing at a rate of 10 °C each minute. Electron ionization (EI) at 70 eV was used to obtain the mass spectra of the chemicals in the samples, and the detector ran in scan mode between 40 and 700 m/z. Starting at 3 minutes, the MS ran 35 minutes with a solvent, and the cut time was roughly 3 minutes.

The essential chemical ingredients were found by comparing mass spectra with those of reference compounds in the National Institute of Standards and Technology's mass spectral database (NIST). Percent peak areas about the overall peak area were used to indicate the relative amounts of each component (IDAN et al., 2015).

2.16. Cell line culture

Human brain cancer A172 and Pancreas Cancer P53 Cell Lines were stored in the vapor phase of liquid nitrogen at a temperature below -130 C° in frozen vials (LI et al., 2014). The frozen cell line vials were stored in the Tissue Culture Laboratory in the Center for Natural Product Research and Drug Discovery, Department of Pharmacology, Faculty of Medicine, the University of Malaya Kuala Lumpur.

2.17. Estimation of cytotoxicity of the extract by MTT Assay

A 96-well plate was used for growing the cells ($1x10^4$ to $1x10^6$ cells mL^{-1}) with a final volume of 200 mL $well^{-1}$. The plate was incubated for 24 hours at 37°C with 5% CO_2 under

sterile parafilm cover. Following the incubation period, the medium was taken out, and 200.0 mL of the pomelo peel extract serial dilution (25, 50, 100, 200, and 400 µg mL^{-1}) was added to the well.

At every concentration, triplicates and controls have been carried out. For 24 hours, plates were incubated at 37°C with 5% CO_2. Phosphate buffer solution was used twice to wash the cells in the well. Each well was filled with 20 µL of the MTT staining solution, and the plate was then incubated at 37 °C. Following 4 hours, every well-received 100 µL of dimethyl sulfoxide (DMSO) was used to dissolve the formazan crystals, and the absorbance was measured at 575 nm. For every set of replicates, the mean absorbance was computed. Equation 1 was utilized to determine the percentage of viable cells after exposure to pomelo peel extract.(TRANCHIDA et al., 2012).

$$\text{Cell viability } \% = \frac{\text{absorbance of treated cells}}{\text{absorbance of control}} \times 100 \qquad (01)$$

2.18. Multi-parameter cytotoxic assay

Five orthogonal cell health parameters for human pancreas cancer Capan-2 were measured using the multi-parameter cytotoxicity test following in vitro exposure to pomelo peel extract. The criteria included viability cell count, cell membrane permeability, total nuclear intensity, mitochondrial membrane permeability, and cytochrome C release.

Following a 24-hour exposure to varying quantities of pomelo peel extract, the treated Capan-2 cells were subjected to 30 minutes at 37 °C of staining solution (MMP dye + permeability dye). Before being probed for 60 minutes with primary cytochrome C antibody and secondary Daylights 649 conjugated goat anti-mouse IgG, cells were fixed, permeabilized, and blocked. Thermo Scientific, USA's Array Scan HCS analyzer was used to examine each plate.

2.19. Statical analysis

To determine whether group variance was significant, a one-way analysis of variance (ANOVA) was carried out. GraphPad Prism v. 6 was used to perform statistical significance tests and express the data as mean ± Standard Deviation (SD).

3. RESULTS

The pomelo plant (*Citrus maxima* L.) was collected from the markets of Hilla City. After obtaining sufficient quantities of peels, they were treated to obtain the aqueous extract, and the study was completed. The extract was treated with a group of treatments to conduct preliminary chemical analysis and identify the important and effective compounds in the peel extract (Table 1).

While the pomelo peel has a wide range of chemical constituents, the main subject of this article is its secondary metabolites. Pomelo peels contain phenolics, steroids, coumarins, phenylpropanoids, flavonoids, and essential oils (MOH; SITI,2024).

The identification and extraction of flavonoid components from pomelo peels have been the subject of numerous investigations. The methods for obtaining extracts include using solvents like ethanol, methanol, or water for extraction (KHAN et al., 2018; SINGH, 2015). Numerous other studies on pomelo peels have also detected the coumarin component. Solvent extraction is used to acquire the chemical (ASHWIN et al., 2024; MOH; SITI, 2024). The pomelo peel contains phenolic chemicals such as gallic acid, ellagic acid, methyl 4-hydroxybenzoate, and 4-hydroxybenzaldehyde (GINOVYAN et al., 2017; MOH; SITI, 2024).

Table 1. Preliminary chemical analysis of pomelo peel extract.
Tabela 1. Análise química preliminar do extrato da casca de pomelo.

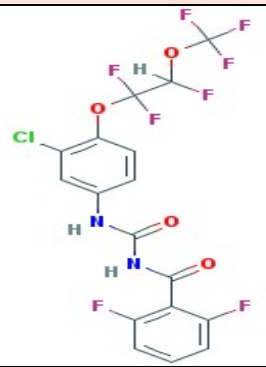
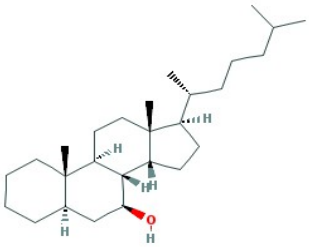
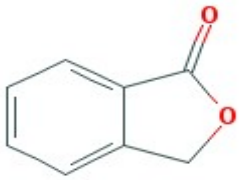
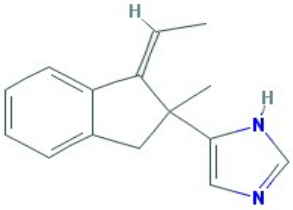
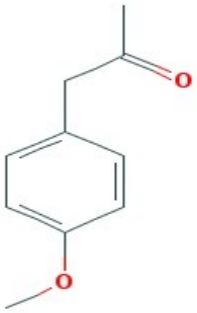
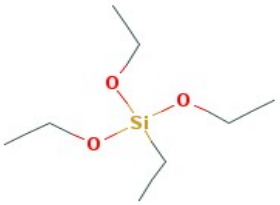
No.	Test for	Test Name	Reagents	Indication of reaction	Result
1	Alkaloid	a. Dragendroff,s	Bismuth nitrate + glacial acetic acid + potassium iodide	Orange coloration	+
		b. Wagner	I+ KI + D.W.	Reddish brown precipitates	+
		c. Mayer	$HgCl_2$ + KI + D.W.	White precipitates	+
2	Phenols	Ferric chloride	Ferric chloride 1%	Bluish-green color	+
3	Flavonoids	Lead acetate	Lead acetate	white precipitate	
4	Tannins	ferric chloride alcoholic	10% ferric chloride alcoholic	Dark blue or greenish grey	+
5	Coumarins	UV light	NaOH + Plant extract + UV light	Green color	+
6	Glycosides	Benedict reagent	Benedict reagent	Pink or red pellet	+
7	Saponin	Foam	Plant extract + D.W.	1 cm of foam	+
8	Resin	HCl	HCl 4%	Turbidity	+
9	Carbohydrates	a. Molisch's	naphthalol in 95% alcohol	Reddish brown ring	+
		b. Fehling's	Copper sulphate + potassium sodium tartrates + NaOH	Reddish brown precipitate	+
10	Terpenes	Liebermann- Burchard test	Chloroform + acetic acid + conc. H_2SO_4	Dark pink/green	+
11	Essential oils	UV light	Extract Saturated filter + UV light	Bright pink	+
	+ = positive result				

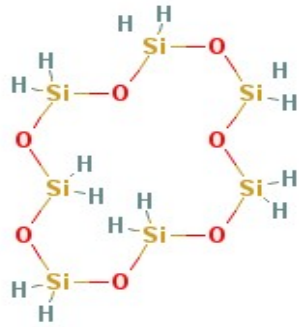
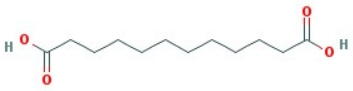
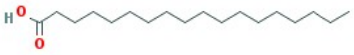
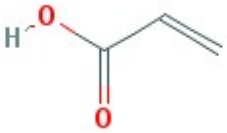
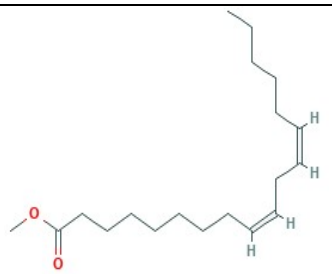
3.1. GC-MS analysis for chemical components of pomelo peels watery extract

The chemical compounds in the pomelo peels have been identified using GC-Mass technology. Table 2 and Figure 1 illustrate each constituent's chemical composition, molecular weight, and chemical structure through its retention duration. The GC-Maas test confirmed the chemicals' presence in the preliminary analysis of chemical compounds; the GC-Mass test verified the presence of allergens, steroid coumarin, phenol, long-chain fatty acid, and dicarboxylic acid. According to GC-MS data, pomelo peels have substantial quantities of the steroid's phenol and

coumarin. Compared to other citrus juices, pomelo juice exhibited significantly greater levels of total phenolic, vitamin C, carotenoid, and δ-tocopherol. Furthermore, He et al. (2019) found that pomelo juice has greater antioxidant activity than citrus juice. In essential oils extracted from the peel and flowers of the Thai pomelo cultivar known as Khao-yai, phenol is the most prevalent chemical (THAVANAPONG et al., 2010).

Table 2. GC-Mass analysis of Pomelo peels watery extract.
Tabela 2. Análise de CG-massa do extrato aquoso da casca de pomelo.

No	Phytochemical Name	Chemical Formula	RT (min)	Molecular Weight (g mol^{-1})	Chemical structure	Nature of compound
1	1,1,1-trichloro-2,2-bis ethane	$C_{17}H_9ClF_8N_2O_4$	3.515	492.706		Phenol
2	Cholestan-7-ol	$C_{27}H_{48}O$	10.38	388.68		Steroid
3	1(3H)-Isobenzofuranone	$C_8H_6O_2$	10.38	134.134		Coumarin
4	1- Cyclohexyl dimethylsilyloxyoctade	$C_{18}H_{122}N_2$	11.141	266.388		Allergens.
5	Propenone	$C_{10}H_{12}O_2$	12.928	164.204		Simplest ketone
6	Silane,triethoxyethyl	$C_8H_{20}O_3Si$	12.928	192.33		Precursor of silicon

7	Cycloheptasiloxane	$H_{14}O_7Si_7$	14.031	322.7		silicon-based cyclic compound
8	Dodecanedioic acid	$C_{12}H_{22}O_4$	16.504	230.304		Dicarboxyli c acid
9	Octadecanoic acid	$C_{18}H_{36}O_2$	16.922	284.484		long-chain fatty acid
10	2-propenoic acid	$C_3H_4O_2$	18.624	72.063		unsaturated Carboxylic acid
11	Hexadecanoic acid	$C_{16}H_{32}O_2$	24.786	256.43		fatty acid
12	Pentadecanoic acid	$C_{15}H_{30}O_2$	24.786	242.403		fatty acid
13	Linoleic acid	$C_{19}H_{34}O_2$	27.971	294.479		doubly unsaturated fatty acid
14	Nonacosane	$C_{29}H_{60}$	44.953	408.799		straight-chain hydrocarbon

The cytotoxicity regarding the extract was assessed with the use of the MTT(3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) assay against the human brain cancer A172, pancreas cancer cell lines Capan-2, and normal human dermal fibroblasts HdFn. The extract was applied to the cell lines at varying quantities: at concentration levels of 25, 50, 100, 200, and 400 µg mL^{-1}, the extract was more effective against cancer cells than normal cells. With an IC50 of 994.826 µg mL^{-1} in Figure 2, the extract had very little effect on normal cells, but it had a considerable effect on cancer cells, and the effect grew with increasing concentration. The vitality of A172 cells at a concentration of 200 µg mL^{-1} was 47.56%, while 41.70% at a concentration of 400 µg mL^{-1} with IC50 = 265.396 µg/ml as cleared in Table 3 and Figure. As well as its effect on Capan-2 at a concentration of 200 µg mL^{-1} was 40.89 %, while it was 35.26 % at a concentration of 400 µg mL^{-1} with IC50 = 192.247 µg mL^{-1}, as shown in Table 3 and Figure 4. The effect of the extract was on more than one person, which may be due to physiological reasons related to tissue elasticity.

Volatile oils (Tranchida et al., 2012), alkaloids (He et al., 2011), coumarins and sterols (Tahsin et al., 2017), flavonoids, and limonoids (Sun et al., 2019) are the identified chemical constituents of plants belonging to this genus. These substances have biological anti-cancer activity (VISALLI et al., 2014). The watery pomelo peel extract had a potent cytotoxic impact on human tumor cells. SAID et al. (2019) found that the oil had a less cytotoxic effect on human tumor cells.

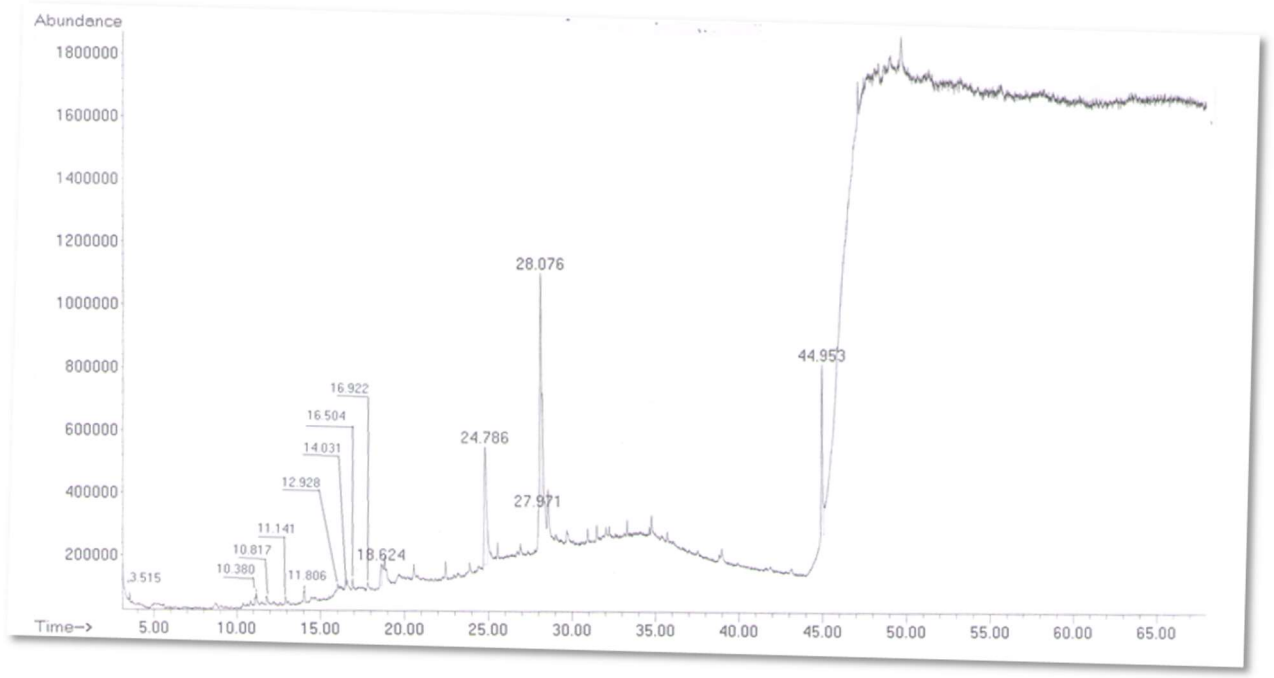


Figure 1. GC-MS chromatogram shows the different peaks of the constituents of pomelo peel watery extract.
Figura 1. Cromatograma de GC-MS mostrando os diferentes picos dos diferentes constituintes do extrato aquoso da casca de pomelo.

Table 3. Cytotoxicity effect of pomelo peels watery extract.
Tabela 3. Efeito citotóxico do extrato aquoso da casca de pomelo.

Pomelo peels Extract concentration (µg mL^{-1})	Cell viability % (mean ± SD)		
	HdFn	A172	Capan-2
25	94.63 ± 0.35	82.48 ± 4.54	79.43 ± 3.54
50	93.71 ± 0.94	73.14 ± 1.44	62.65 ± 3.25
100	87.61 ± 5.15	60.87 ± 6.02	50.19± 2.47
200	82.71 ± 0.89	47.56± 5.30	40.89 ± 2.21
400	70.56± 1.95	41.70 ± 1.91	35.26 ± 1.07

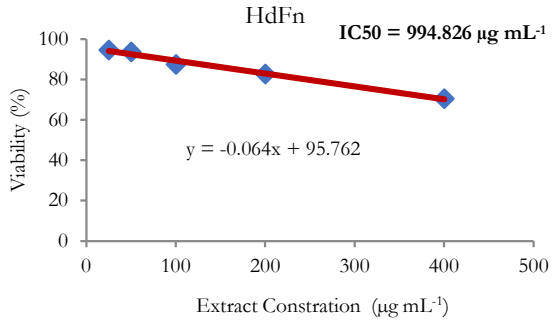


Figure 2. Cytotoxicity effect of pomelo peels watery extract against HdFn.
Figura 2. Efeito citotóxico do extrato aquoso da casca de pomelo contra HdFn.

Pomelo contains bioactive chemicals that may scavenge free radicals and induce apoptosis to prevent certain types of cancer (POULOSE et al., 2005). A concentration was linked to cytotoxicity, suggesting that Pomelo peel watery extract could induce apoptosis, revealing that apoptosis was the main mechanism causing cell death. Cytotoxicity was related to a concentration suggesting that Pomelo peels' watery extract could induce apoptosis, which is mostly responsible for cell death (CARDILE et al., 2015; ABRAHAM et al., 2008).

A few authors studied the cytotoxicity effect of pomelo using five human tumor cell lines: human breast adenocarcinoma MCF7, human hepatoma HepG2, human lung carcinoma A549, human cervical cancer Hela, normal human non-tumorigenic epithelial breast MCF10A cells, and human laryngeal carcinoma HEp2 cells. Those cells cleared a high cytotoxicity effect against these tumor cells (POULOSE et al., 2005; HAFIDH et al., 2018).

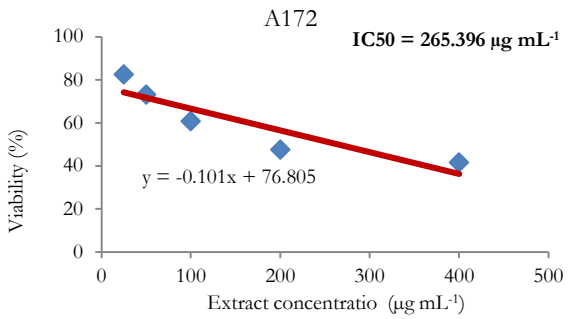


Figure 3. Cytotoxicity effect of Pomelo peels watery extract against A172.
Figura 3. Efeito de citotoxicidade do extrato aquoso da casca de pomelo contra A172.

Research indicates that the extract could trigger the expression of p52, which controls the expression of the pro-apoptotic Bax gene and the cell cycle regulator p21 gene. This ultimately results in the G1 phase arrest of HT-29 cells, the release of cytochrome C, and apoptosis (YUAN et al., 2013).

Additionally, the extract may function by blocking the actions of topoisomerase I and II, an enzyme that fragments DNA and promotes cell growth and proliferation inhibition (SAPKOTA et al., 2012; PROKHOROVA et al., 2015).

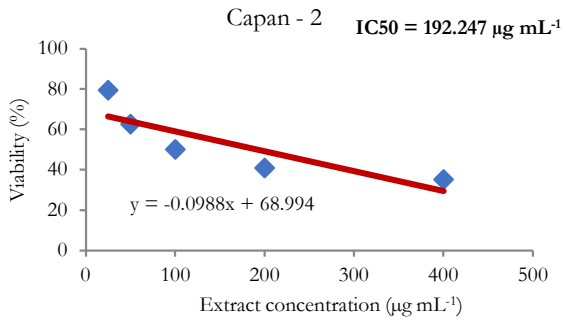


Figure 4. Cytotoxicity effect of Pomelo peels watery extract against CaPan-2.
Figura 4. Efeito de citotoxicidade do extrato aquoso da casca de pomelo contra CaPan-2.

3.2. High Content Screening (HCS) cytotoxicity evaluation

With the use of the parameters of cell viability (VCC), membrane permeability (CMP), nuclear density (TNI), mitochondrial membrane potential (MMP), and cytochrome C release (CC), HCS assessed the cytotoxicity of pomelo peel extract in vitro to identify changes in CaPan-2 cells. Untreated cells were used as a comparison for these parameters. The vitality of CaPan-2 cells was dramatically reduced at 100, 200, and 400 µg mL^{-1}, although only a modest reduction was seen in cells treated with 25 and 50 µg mL^{-1}. With a rate at which the quantity of live cells decreases. The plant extract was applied to CaPan-2 cells at 100, 200, and 400 µg mL^{-1}. The results of the assessment of cell viability (VCC) indicated in Table 4 demonstrated a significant decrease in the vitality of the cells when compared with the negative control (untreated cells). There was no discernible difference at the lower extract amounts, 25 and 50 µg mL^{-1}. Total nuclear density is the second indicator (TNI).

Table 4. The cytotoxicity of pomelo peel extracts in vitro by HCS.
Tabela 4. Citotoxicidade de extratos de casca de pomelo in vitro por HCS.

	HCS Parameters (mean ± SD)				
Concentration (µg mL^{-1})	Valid Cell Cont.	Total Nuclear Intensity	Cell membrane permeability	Mitochondrial membrane permeability	Cytochrome C
Control untreated Cell	3244 ± 321.7	428 ± 4.35	133 ± 14.23	572 ± 13.53	410 ± 10.43
25	3236 ± 341.8	413 ± 13.82	132 ± 6.43	525 ± 15.76	409 ± 9.75
50	3128 ± 326.4	446 ± 4.56	145 ± 12.76	488 ± 11.84	397 ± 11.36
100	2185 ± 212.8	571 ± 15.84	141 ± 16.32	397 ± 9.72	587 ± 11.82
200	1947 ± 197.3	755 ± 36.72	259 ± 13.74	366 ± 12.04	683 ± 13.63
400	1324 ± 138.6	942 ± 45.11	305 ± 17.62	309 ± 11.83	813 ±15.42

According to studies by PROKHOROVA et al. (2015), nuclear morphology is a telltale sign of programmed cell death in the nucleus. Table 4 results demonstrate how the extract affected MCF cells. Compared to the negative control, CaPan-2cells exposed to high extract concentrations of 100 and 200 micrograms mL^{-1} markedly increased cell nuclear formation. Changes in cell nuclear density are closely connected with apoptotic features like chromatin condensation, cell DNA fragmentation, bleeding, and shrinkage (JAMALZADEH et al., 2017).

The permeability of the cell membrane is the third indicator (CMP). The cell membrane density was considerably altered in CaPan-2cells treated with 200 and 400 micrograms/ml of extract compared to untreated cells, as demonstrated by the data displayed in Table 4 and Table 3. The permeability of the membrane was dramatically reduced. The permeability of the cell membrane is a measure of the integrity of the cell; a decrease in permeability suggests a permeability defect, which has a major impact on the cell's vitality (THAVANAPONG, 2010). The strength of the mitochondrial membrane (MMP) is the fourth indicator. Using the dye MMP, which only enters active and functioning mitochondria and builds up inside the inner mitochondrial membrane, the impact of pomelo peel extract on the integrity of the mitochondrial membrane was investigated (SEKI; RUTZ, 2018). The outcomes are displayed in Table 4. As the extract concentration increased, the strength of the mitochondrial membrane decreased dose-dependently. Maximum decrease was contrasted with cells not treated at 400 and 200 µg mL^{-1}; no statistically significant changes were observed with the extract treatment.

A key component of programmed cell death is the shape and strength of the cellular mitochondria's membrane; thus, membrane strength changes directly cause cells to undergo programmed cell death (ARBAB et al., 2013). Depolarization of the membrane and its weakening are signs of a malfunctioning mitochondrial function, which is progressively compromised. Zhang et al. (2015) found that drug toxicity and this characterization of cell death signals are effective.

Only at concentrations of 100, 200 and 400 µg mL^{-1} did the results in Table 4 demonstrate a substantial increase in the amount of cytochrome released from mitochondria compared to the negative control (untreated cells). At the concentrations, no discernible variations were found. As the cytosol and cytochrome C activate caspase, a family of lethal proteases, by establishing a complex with Apaf1 (apoptotic protease activating factor), procaspase-9 and ATP or dATP are released. The lowest is 25 and 50 µg mL^{-1}. Bcl-2 family proteins are responsible for controlling mitochondrial cytochrome C. Apoptotic factor, cytochrome C, and some peroxidase proteins that are released into the cell by the cytosol are among the proteins that are typically sequestered between the mitochondrial membranes during the process of cell death. This event can cause necrosis by mitochondrial damage or apoptosis through cytochrome c-dependent caspase activation, depending on the stimuli (GOLDSTEIN et al., 2000).

4. CONCLUSIONS

Cancer is a modern problem that threatens human lives, and attempts to eliminate it are continuing. During this study, the extract of pomelo (*Citrus maxima* L.) peels demonstrated high efficiency in eliminating cancer by studying its effect on human brain cancer A172 and pancreas cancer Capan-2 cell lines. This was done after a preliminary chemical analysis of the most important compounds in the pomelo (*Citrus maxima* L.) peel extract.

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Data availability: The corresponding author can obtain study data by e-mail.

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