



A comparative study of antioxidative, antimicrobial, and anticancer activities of ginger and lemon peel extracts

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ABSTRACT

Owing to their great quantity of various phytochemicals, ginger, and lemon peel extracts exhibit potent antioxidant, antimicrobial, and anticancer properties. The present study was designed to investigate the phytochemical composition of ginger and lemon peel aqueous and ethanolic extracts, their potential antimicrobial activity, and anticancer properties. Ginger peel powder possesses the highest phytochemical content compared to lemon peels. Furthermore, the maximum phenolic and flavonoid contents of ginger were extracted in ethanolic extracts, which were documented as 20.65 mg GAE/g and 12.96 mg QC/g compared to lemon peel extracts. Additionally, ethanolic and aqueous ginger peel extracts exhibited superior antioxidant activity (96.91 and 95.77%, respectively) compared to lemon peel extract. HPLC fractionation further revealed the diverse phenolic and flavonoid compounds present in both peels, with ethanolic extracts consistently outperforming their aqueous ones. The ethanolic extracts of both ginger and lemon peels exhibit a higher degree of antimicrobial activity compared to their aqueous extract. Notably, the ethanolic extract of ginger peels demonstrates remarkable inhibition zones, particularly against gram-positive bacteria and gram-negative bacteria. On the other hand, ethanolic extracts of both peels exhibited potent cytotoxic effects against liver and colon carcinoma cells, with ethanolic extract of ginger peel showing superior anticancer potential with IC_{50} (4.7 and 5.2 $\mu\text{g/ml}$, respectively). Apoptotic and necrotic cell analyses underscored the ability of ginger peel ethanolic extract to induce a higher level of cancer cell apoptosis while minimizing necrosis. Therefore, our study lies in the distinct phytochemical profile of ginger and lemon peel extracts, emphasizing the higher phenolic and flavonoid contents in ethanolic extracts and their effect as antimicrobial and anticancer activity.

Keywords: *Ginger peels, Lemon peels, Antioxidants, Antimicrobial Activity, Cancer cell lines, Apoptosis, Necrotic*

1. Introduction

Oxidative stress results from an imbalance between the creation of reactive oxygen species (ROS) and elimination, contributing to health problems like cancer, heart disease, aging, and neurodegenerative issues[1]. ROS is produced internally due to psychological stress, tissue hypoxia resulting from the compromised blood supply, microbial infections, malignancy, and the aging process and externally (radiation, pollutants)[2]. Moreover, ROS can damage macromolecules such as DNA, proteins, and lipids, resulting in modifications to genetic material that can potentially induce the development of cancer [3].

Cancer, a complex and multifactorial disease, involves uncontrolled cell growth and proliferation that can invade surrounding tissues and even spread to other body parts. While various genetic and environmental factors contribute to cancer development, oxidative stress has emerged as a critical player in the initiation and progression of cancer. Oxidative DNA damage can cause mutations and play an important role in the initiation and progression of carcinogenesis [4][5]. Therefore, the antioxidant balancing role against elevated ROS levels is vital for many diseases, including various cancers [6].

In this context, the role of phytochemical compounds found in wasted food takes on significant importance due to their role as antioxidants which destroy the overproduction of free radicals[7]. Different scientific studies reveal that various plant parts (bark, pods, seeds, pods, latex, fruit, flowers, stems, leaves, ligules, bark, stems, roots, etc.) containing phytochemicals exhibit different biological activities[8]. Furthermore, phytochemicals are known for their potential to combat oxidative stress and modulate various cellular processes. These compounds, including flavonoids, polyphenols, carotenoids, and sulfur-containing compounds, possess antioxidant properties that help neutralize ROS, thereby reducing oxidative damage to cells and biomolecules[9].

Citrus fruits from the "*Rutaceae*" family yield 102 million tons annually[10]. Citrus limon is rich in nutrients (vitamin C and minerals) and antioxidants like phenolic compounds, ascorbic acid, essential oils, and carotenoids, combating cellular oxidative damage[11]. Remarkably, citrus peels, comprising 50–60% of total fruit weight, possess untapped potential, which exhibits antimicrobial, anti-inflammatory, anticancer, and cardio-protective properties[12][13]. Moreover, Citrus peel extract shows high antioxidant activity compared to synthetic antioxidants[14].

Ginger (*Zingiber officinale*) is well-known as a spice and traditional medicine system, especially for its bioactive compounds [15]. Notably, ginger peels, often discarded, possess significant antioxidant properties due to phenolic compounds, flavonoids, and essential oils content. These qualities enable them to combat free radicals, potentially benefiting health by mitigating oxidative stress, inflammation, and chronic diseases. Research suggests ginger peel extracts hold promise against cardiovascular issues, cancer, and neurodegenerative disorders, alongside antimicrobial and anti-inflammatory effects [16].

The current work lies in the comprehensive exploration of the phytochemical and antimicrobial activities of lemon and ginger peels . By focusing on the often-overlooked peels of these widely consumed foods, we aim to contribute novel insights into their therapeutic potential against oxidative stress-related health challenges especially anticancer agents including apoptosis and necrosis cells.

2. Material and methods

2.1. Raw materials

- The fresh and mature Eureka lemon (*Citrus limon*) was obtained from Research and Production Station of National Research Centre, El-Nubaria district, El-Behira Governorate, Egypt.

- The fresh and mature ginger (*Zingiber officinale*) was obtained from the local market in Cairo, Egypt. Lemon and ginger were cleaned with distilled water and manually peeled using a sharp knife. The peels were washed, dried in an oven at 50 °C, crushed using a pestle and mortar, a high-speed laboratory blender, and sieved with a mesh size of 20 to 30, producing a finely separated powder[17].

2.2. Chemicals and reagents

The reagents utilized in the study included 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid, and quercetin. Muller Hinton agar, peptone water, and nutrient broth were purchased from Sigma-Aldrich Chime in Steinheim, Germany. All chemicals and solvents employed in the research were obtained from MERCK in the USA.

2.3. Preparation of plant extracts

2.3.1. *Water extract*: Lemon and ginger peel powder (100 g) was macerated in 1000 ml of cold water at room temperature and stirred continuously for 24 hours. The extract was filtered using muslin and Whatman No. 1 filter paper.

2.3.2. *Ethanol extract*: Lemon and ginger peel powder (100 g) was macerated 3 times in 80% (v/v) ethanol and mixed using magnetic stir at room temperature for 2 h., then allowed to stand in a refrigerator at 4±1 °C

for 24 h. All extracts were concentrated in a rotary evaporator (Stuart Rotary Evaporator Model RE300) at $40 \pm 1^\circ\text{C}$ and finally freeze-dried (Snijders Scientific type 2040); the lyophilized materials were stored at $4 \pm 1^\circ\text{C}$ for further use[18].

2.4. Determination of total phenolic and flavonoid compounds

The determination of total phenolic compound content followed the method reported by **Maurya and Singh** [19], 1 ml of sample extract, 5 ml Folin Ciocalteu reagent (1:10 v/v) followed by 4 ml of sodium carbonate (7.5%), and mix for 15 seconds with vortex, then kept in the dark place for 30 min. The absorbance was recorded at 760 nm using a spectrophotometer, and the finding results were expressed as mg Gallic acid equivalents (GAE) / gram of the sample weight.

Total flavonoid content was quantified following the method reported by **Jia et al.**[20], 0.5 ml of sample extract, 2 ml of distilled water, and then add 0.15 ml of NaNO_2 (5%) incubation for 6 min, 0.15 ml of AlCl_3 (10%) and allow to stand for 6 min before adding 2 ml of NaOH (4%,). Then, complete the volume to 5 ml with distilled water. The absorbance was recorded at 510 nm. The finding results were expressed as mg of Quercetin equivalents (QC) /gram of the sample weight.

2.5. Fractionation and identification of phenolic and flavonoid compounds

The phenolic and flavonoid components were identified using HPLC. The sample, weighing one gram, was mixed with methanol and then subjected to centrifugation and filtration using a 0.2 m Millipore membrane filter to isolate phenolic chemicals. The solution that had been filtered (1-3 mL) was introduced into an HPLC system (Hewlett Packard, series 1050) equipped with a UV detector specifically designed to detect phenolic acids set at a wavelength of 280 nm[21]. Concurrently, the identical sample was introduced into an HPLC system equipped with a UV detector set at 333 nm, specifically designed to detect flavonoid compounds. The concentrations of components were calculated using Hewlett Packard Software through data analysis, which involved considering retention time and peak area[22].

2.6. Determination of antioxidant activity

The antioxidant activity of lemon and ginger peel powder and their extracts was evaluated by the 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity according to the colorimetric assay of [23]. 100 μl of sample extract was mixed with 4 ml of DPPH solution ($6 \times 10^{-5}\text{M}$) and incubated in the dark for 30 min, then measured at 517 nm The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of [24].

$$\text{Inhibition\%} = (\text{Ac (0)} - \text{AA (t)}) / \text{Ac (0)} \times 100$$

Where:

Ac (0) is the absorbance of DPPH as control at 0 min.

AA (t) is the absorbance of samples after 30 min.

2.7 Antibacterial activity

The antibacterial activity of lemon and ginger peel aqueous and ethanolic extracts was detected using the agar well diffusion technique according to the method reported by **Patel et al.**[25]. At the same time, the control wells also contained pure solvents as the negative control. The antimicrobial activity against the gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium*, as well as the gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*, was determined by measuring the zone of inhibition (mm) [26].

2.8. Antifungal activity

The antifungal activity of lemon and ginger peel aqueous and ethanolic extracts was assessed using the potato dextrose agar well diffusion technique. Control wells containing pure solvents as negative control were also involved. The antifungal activity against *Aspergillus flavus* and *Candida albicans* was calculated by measuring the zone of inhibition (mm) at intervals every 24 hrs[27].

Cytotoxic and antitumor activities of selected plant extracts

The cytotoxicity or anticancer activity of lemon and ginger peel powder and their extracts was estimated using the MTT test on two tumor cell lines HT-29 colon carcinoma cells and HepG2 liver cancer cells, which were obtained from National Cancer Institute Laboratories, Cairo University, Egypt. The cells were cultured in DMEM medium enhanced with 10% HI-FBS, and experiments were done in triplicate. Cells were incubated for 72 hours, and then MTT was added to each well. The extent of MTT reduction was measured spectrophotometrically, and cytotoxicity was expressed as the different types of extract concentration, which inhibited cell growth by 50% (IC₅₀). The IC₅₀ values were assayed using Graph Pad Prism 7 computer program (GraphPad Software, S. Diego, CA, USA).

2.9. Apoptosis and necrosis cells assay

The Annexin V-FITC apoptosis detection kit and two fluorescent channels in flow cytometry were used to assess the populations of apoptotic and necrotic cells. Following treatment with test compounds or doxorubicin for different times, cells were gathered, washed with ice-cold PBS, and subjected to an incubation period with Annexin V-FITC/PI solution for 30 minutes in a dark environment at room temperature. The stained cells were analyzed using a flow cytometer, with the FITC and PI fluorescent signals detected using FL1 and FL2 signal detectors, respectively. The quantification of cells positively stained with FITC and/or PI was conducted through quadrant analysis, facilitated by ACEA NovoExpress™ software (ACEA Biosciences Inc., San Diego, CA, USA).

2.10. Reading (Biological Significance of each phase):

Early apoptosis phase (Q4), Late apoptosis phase (Q2), Necrosis phase (Q1).

2.11. Statistical analysis:

Data analysis was performed using SPSS version 22. Results were shown as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) was conducted, followed by the LSD post hoc test. Pearson correlation coefficient was computed to determine correlations between variables. A significance level of $p < 0.05$ was deemed as statistically significant.

Results

The total phenolic and flavonoid contents of lemon and ginger peel powder and their extracts

Table 1. The total phenolic and flavonoid contents of lemon and ginger peel powder and their extracts

Samples	Constituents mg/g		Total phenolic	Total flavonoids
Ginger	Powder		13.83 ^c \pm 0.14	3.32 ^{cd} \pm 0.20
	Ethanol		20.65 ^a \pm 0.19	12.96 ^a \pm 0.25
	Aqueous		16.18 ^b \pm 0.16	7.94 ^b \pm 0.23
Lemon	Powder		1.58 ^e \pm 0.02	1.37 ^d \pm 0.20
	Ethanol		9.89 ^d \pm 0.28	7.03 ^b \pm 0.15
	Aqueous		8.49 ^d \pm 0.12	4.98 ^c \pm 0.01

The different letters indicate statistically different means according to LSD post hoc test

Antioxidant activity of lemon and ginger peel powder and their extracts using DPPH

Table 2. Antioxidant activity of ginger and lemon peel powder and their extracts using DPPH

Samples	Powder	Ethanol	Aqueous	p-value
Ginger	80.96 ^c \pm 0.50	92.91 ^a \pm 0.38	54.77 ^e \pm 0.83	$p < 0.001$
Lemon	75.58 ^d \pm 1.00	86.29 ^b \pm 1.40	47.56 ^f \pm 0.29	$p < 0.001$
p-value	$p < 0.001$	$p = 0.124$	$p < 0.001$	

The different letters indicate statistically different means according to LSD post hoc test. Small letters for horizontal comparisons, capital letters for vertical comparisons.

Fractionation of phenolic compounds of lemon and ginger peel powder and their extracts

Table 3. Fractionation of phenolic compounds of lemon and ginger peel powder and their extracts

Phenolic compounds mg/100g	Ginger peel			Lemon peel		
	Powder	Ethanol	Aqueous	Powder	Ethanol	Aqueous
Pyrogallol	114.39	395.96	260.38	23.77	157.95	130.57
Gallic acid	34.71	54.76	39.36	3.83	24.86	7.44
Catechol	19.31	30.69	23.66	0.72	7.03	2.98
4-Aminobenzoic	6.4	10.35	8.26	0.55	2.87	0.68
Catechein	15.82	227.19	115.54	12.93	49.11	34.07
Chlorogenic acid	35.27	51.02	48.89	5.77	36.28	17.03
Benzoic acid	85.90	210.79	108.85	6.21	10.3	7.32
Vanillic acid	4.39	8.99	6.21	4.74	13.78	9.03
Caffeic acid	5.19	7.07	5.87	5.87	11.95	9.11
Ferulic acid	16.87	21.23	18.8	4.4	9.07	5.77
Salicylic acid	19.55	37.11	28.22	2.45	10.65	8.13
Ellagic acid	50.89	309.31	238.45	3.77	10.71	9.38
Coumarin	9.17	18.61	16.44	3.95	7.46	14.13
Caffeine	37.53	140.5	79.02	4	6.43	5.95
Protocatechuic acid	4.96	58.13	29.25	3.3	7.08	5.32
Iso-Ferulic acid	9.17	12.1	10.12	1.89	14.2	9.28
α -Coumaric acid	10.15	20.3	15.87	0.85	63.7	32.88
3,4,5-methoxy-cinnamic acid	7.33	18.14	13.47	5.78	14.41	10.65
Total	487.0	1632.25	1066.66	94.67	462.74	319.72

Fractionation of flavonoid compounds of lemon and ginger peel powder and their extracts

Table 4. Fractionation of flavonoid compounds of ginger and lemon peel powder and their extracts

Flavonoid compounds mg/100g	Ginger peels			Lemon peels		
	Powder	Ethanol	Aqueous	Powder	Ethanol	Aqueous
Hesperidin	93.25	446.75	160.9	7.01	116.39	95.17
Rosmarinic	1.54	7.94	4.65	2.41	7.69	3.65
Rutin	3.42	18.52	10.6	6.47	55.45	28.8
Apigenin 7-glucose	0.82	4.65	2.92	5.88	18.25	14.34
Quercetrin	2.41	24.37	12.48	3.57	28.21	17.73
Naringin	7.23	60.3	43.54	12.5	63.96	46.03
Naringenin	1.89	31.83	5.04	3.12	3.03	24.07
Quercetin	2.02	42.69	4.54	2.43	8.3	20.4

Kampferol 3-2-p-coumaroylglucose	47.56	154.71	111.85	4.22	12.85	9.99
Kampferol	1.51	7.05	3.85	2.1	13.11	8.93
Apigenin	0.54	2.80	1.52	1.36	4.73	2.55
Hesperitin	2.21	7.05	5.87	18.65	44.27	29.41
Apignin	2.19	6.86	4.58	4.5	7.84	5.11
Total	166.59	815.52	372.34	74.22	384.08	306.18

The antibacterial activity of ginger and lemon peel extracts

Table 5. The antibacterial activity of ginger and lemon peel extracts

Tested Microorganisms		Ethanol extract		Aqueous extract		Control
		Ginger	Lemon	Ginger	Lemon	Gentamycin
Gram Positive Bacteria	<i>Staphylococcus aureus</i> (RCMB 010010)	20	16	7	4	22
	<i>Bacillus subtilis</i> (RCMB 015 (1) NRRL B-543)	18	13	5	3	20
Gram Negative Bacteria	<i>Escherichia coli</i> (RCMB 010052 ATCC 25955)	15	12	4	5	15
	<i>Salmonella typhimurium</i> (RCMB (RCMB 006 (1) ATCC 14028)	17	15	6	5	18

The test was done using the diffusion agar technique. Well diameter 6.0 mm (100 ul was tested)

Positive control for bacteria Gentamycin (4 ug/ml)

The ethanol and aqueous extracts were tested at a 10 mg/ml.

Antifungal activity of ginger and lemon peel extracts

Table 6. Antifungal activity of ginger and lemon peel extracts

Tested Microorganisms	Ethanol extract		Aqueous extract		Control
	Ginger	Lemon	Ginger	Lemon	Ketoconazole
<i>Aspergillus flavus</i> (RCMB 002002)	15	12	9	6	16
<i>Candida albicans</i> (RCMB 005003(1)ATCC 10231)	18	15	10	8	20

The test was done using the diffusion agar technique. Well diameter 6.0 mm (100ul)

Positive control for Fungi Ketoconazole 100 ug/ml

The ethanol and aqueous extracts samples were tested at 10 mg/ml.

Cytotoxicity and anticancer activity of the ginger and lemon peel powder and their extracts on tumor cell lines
IC₅₀ (µg/ml)

Table 7. Cytotoxicity and anticancer activity of the ginger and lemon peel powder and their extracts on tumor cell lines IC₅₀ (µg/ml).

Samples Cells	Ginger peels			Lemon peels		
	Powder	Aqueous	Ethanol	Powder	Aqueous	Ethanol
HT-29	13	10.7	5.2	42.7	30.2	22.5
HepG2	8.7	6	4.7	31.7	25.5	19.5

IC₅₀ is the concentration of extract that affords a 50% reduction in cell growth (After 72 hours of incubation).

HT-29: Human colon cancer cell line.

HepG2: Human liver cancer cell line.

Apoptotic and necrotic cells

Table 9. The apoptotic, necrotic cells and A/N ratio of HC-29 cell line under the effect of ginger and lemon peel powder and their ethanolic extract

Samples		Apoptotic cells %	Necrotic cells%	A/N Ratio%
Ethanol	Lemon	54.0 ±0.92	0.82±0.38	65.85 ±0.05
	Ginger	58.24 ±0.13	1.96±0.09	29.71 ± 0.06
Powder	Lemon	39.07 ±0.55	7.88 ±1.09	4.96 ±2.54
	Ginger	45.62 ±0.38	2.25 ±0.11	20.27 ±3.15
p-value		p <0.001	p <0.001	p <0.001

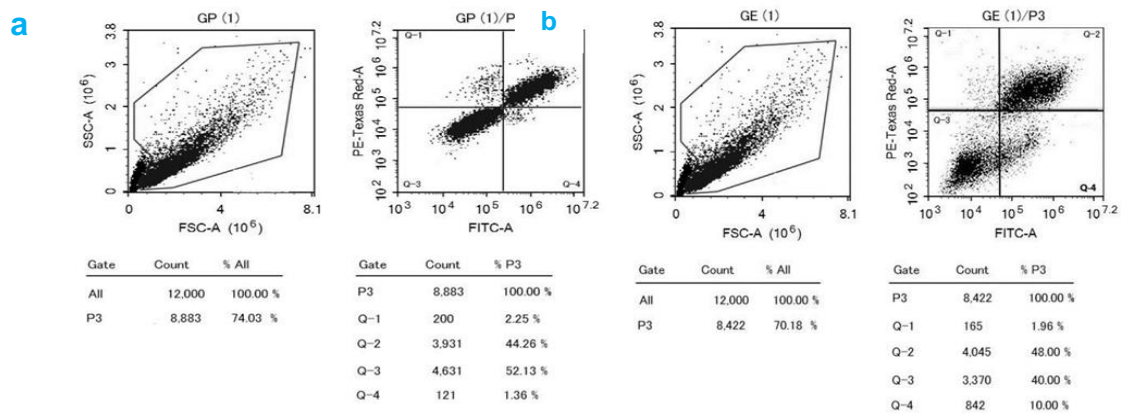


Figure 6. Number and percent of apoptotic (Sum of Q2&Q4) and necrotic cells (Q1) under the effect of a) ginger peel powder and b) the ethanolic extract of ginger.

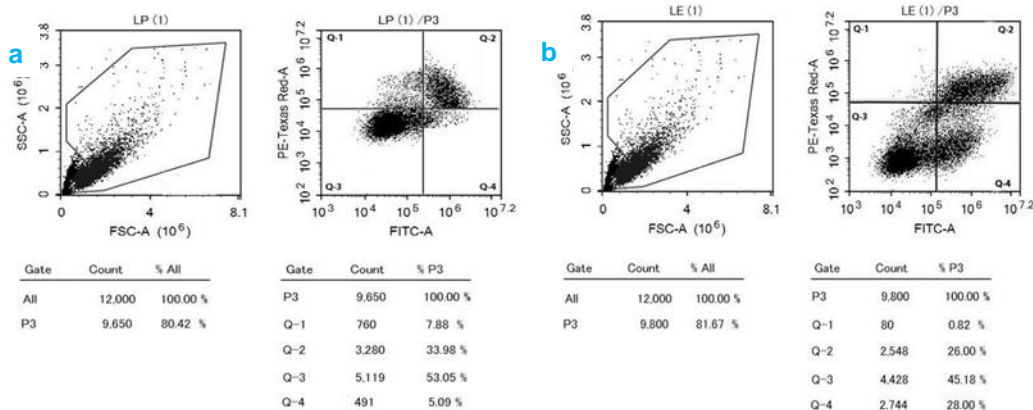


Figure 7. Number and percent of apoptotic (Sum of Q2&Q4) and necrotic cells (Q1) under the effect of a) lemon peel powder and b) the ethanolic extract of lemon against HC-29 cell line.

Discussion

The total phenolic and flavonoid contents of lemon and ginger peel powder and their extracts

The total phenolic content (TPC) evaluation commonly employs the Folin-Ciocalteu (FC) reagent, a broadly utilized method. This procedure generates a redox reagent interacting with polyphenols, resulting in a blue-colored phosphotungstic-phosphomolybdic complex. This complex captures free radicals, allowing for spectrophotometric measurement of reactions [28]. Therefore, the TPC of agro-waste, i.e., lemon and ginger peel powder and their extracts, was determined, and the results are shown in Table (1). Significant difference among all samples was detected. Among raw materials, ginger peel powder exhibited the highest content of TPC (13.83 mg GAE/g), while the lowest was lemon peel powder (1.58 mg GAE/g). On the other hand, both ethanolic and aqueous extracts of ginger peels had the highest content of TPC, which recorded 20.65 and 16.18 mg GAE/g, respectively, compared to ethanolic and aqueous extracts of lemon peels.

Regarding total flavonoid content, ginger peel powder had a higher content of TFC (3.32 mg QC/g) than lemon peel powder which had 1.37 mg QC/g. In contrast, the findings show that both ethanolic and aqueous extracts of ginger peels had the highest content of TFC (12.96 and 7.94 mg QC/g, respectively, compared with ethanolic and aqueous extracts of lemon peels. These results agree with those reported by **Gabr et al.** [29], who noted that ginger ethanolic extract's total phenolic and flavonoid constituents were 26.3 and 9.13 mg/100g, respectively. Also, These results are in agreement with those of **Al-Juhaimi et al.** [30] who reported that total phenolic and flavonoid contents of ethanolic extracts of (Eureka, Citrus limon, Saudi Arabia) peels were (61.22 and 32.70 ± 1.06 mg/g, respectively).

It was observed that the organic solvent (ethanolic extract) was the best extracting method for TPC and TF in fruit peels than water. The increased concentration of phenolic and flavonoid compounds observed in the ethanolic extract, as compared to the aqueous extract, can be attributed to their enhanced solubility in organic solvents. Polyphenolic compounds, possessing a polar nature, readily dissolve in polar solvents such as aqueous methanol or ethanol. On the contrary, their solubility in nonpolar solvents like ether faces difficulties [31].

Antioxidant activity of lemon and ginger peel powder and their extracts using DPPH

Recently, many studies have focused on the role of free radicals in diseases like cancer and vascular conditions. There is increasing interest in natural antioxidants, such as vitamins and polyphenolic compounds, as potential preventive agents of free radicals. Free radicals, characterized by unpaired electrons, are highly reactive and engage in chemical interactions with cellular components in the body. The most significant free radicals in biological systems are termed reactive oxygen species (ROS), including hydroxyl radicals, hydrogen peroxide, and superoxide anions [32].

Table (2) reveals that the antioxidant activity (antiradical properties) of lemon and ginger peel powder and their extracts were compared using stable DPPH free radicals. The results indicate that the difference between the two plants, ginger powder, and their extracts using two extraction methods was statistically different, and they recorded the highest antioxidant activity compared to lemon powder and their extracts. Meanwhile, under the same extraction, ethanol of ginger peels had the highest antioxidant activity ($92.91\% \pm 0.38$) compared with an aqueous extract ($54.77\% \pm 0.83$). On the other hand, the aqueous extract of lemon peel exhibited the lowest antioxidant activity, recorded $47.56\% \pm 0.29$, where both powder and ethanol extract recorded higher antioxidant activity. According to a previous study, ginger peel methanolic extract recorded the highest antioxidant activity compared to potato, garlic, and onion peels [33].

Regarding the composition of different extracts, they exhibit varying profiles of bioactive constituents. Beyond their radical scavenging activity (RSA) and oxidative stability, these extracts' characteristics are influenced by phenolic composition, minor fat-soluble bioactive, and initial hydroperoxide levels. It could be said that the RSA of extracts can be explained as the multiple actions of different endogenous antioxidants. Phenolic compounds and flavonoids, known for their antioxidative role in biological systems, function as scavengers of free radicals and singlet oxygen species [34]. Therefore, antioxidant activities are closely linked to the presence of phenolic compounds [35].

Fractionation of phenolic compounds of lemon and ginger peel powder and their extracts

lemon and ginger peel powder and their extracts were separated by HPLC analysis to identify phenolic compounds, and the results are presented in Table (3). Eighteen phenolic compounds were detected, and pyrogallol was the primary phenolic compound detected in ginger and lemon peel powder, recording 114.39 and 23.77 mg/100g, respectively. Meanwhile, benzoic acid was the second phenolic acid detected in ginger peel powder (85.90 mg/100g) compared to lemon peels which were catechin (12.93 mg /100g). Moreover, gallic, chlorogenic, ellagic, protocatechuic acids, catechol, and caffeine were found in moderate amounts in ginger peel powder compared to lemon peels, which were detected in a small amount.

Among all extracts of ginger and lemon peels, ethanolic extract exhibited the highest content of phenolic acids. Moreover, pyrogallol was the most common phenolic compound in ginger peel ethanolic and aqueous extract (395.96 and 260.38 mg/100g). Notably, ellagic acid was the second identified phenolic acid in ethanolic and aqueous ginger peel extracts (309.31 and 238.45 mg/100g), followed by catechin 227.19 and 115.54 mg/100g). On the other hand, catechol, gallic, chlorogenic, salicylic acid, and protocatechuic acids were found in moderate amounts. Meanwhile, 4-aminobenzoic, vanillic, and caffeic acids were detected in small quantities.

Regarding lemon peel extracts, pyrogallol was the predominant phenolic acid in ethanolic and aqueous extracts, which had 157.95 and 130.57 mg/100g, respectively. In contrast, the second phenolic acid was α – coumaric acid for ethanolic extract (63.7 mg/100g) and catechin for aqueous extract (34.07 mg/100g). Gallic and chlorogenic acids were detected in moderate values in ethanolic and aqueous extracts.

Concomitantly, ginger and lemon peel extracts, mainly the ethanolic extract, displayed increased phenolic acid content during extraction. Ethanolic extracts of ginger and lemon peels had higher phenolic content (1632.25 and 462.74 mg/100g, respectively) compared to aqueous extracts (1066.66 and 319.72 mg/100g, respectively), and their powder (487.0 and 94.67 mg/100g). These results are similar to those of **He et al.** [36], who reported that chlorogenic, caffeic, and ferulic acid in citrus peels ranged from 8.8 to 18.7, 4.5 to 29.9, and 14.4 to 97.8 μ g/g, respectively, as well as, lemon peel contained higher amounts of ferulic, p-coumaric and caffeic acids (44.9, 34.9 and 14.2 mg/100 g, respectively) [37]. Otherwise, these results are similar to those of **Albaridi et al.** [38], who said that the phenolic acids contents of the water extract of ginger:thyme: coriander mixture using HPLC were chlorogenic, ferulic acids, coumarin, and salicylic acid) (48.63, 225.71, 31.5, and 907.16 mg/100g, respectively). As shown in Table 1, the concentration of phenolic compounds was significantly greater in ethanol extracts than in aqueous extracts; this difference can be attributed to the greater polarity of ethanol, which increases extraction capabilities, yielding more phytochemicals and active constituents from the basic materials [39].

Fractionation of flavonoid compounds of lemon and ginger peel powder and their extracts

Flavonoids are natural phenolic compounds with a unique structure of two aromatic rings (A & B) linked by a three-carbon bridge (C6–C3–C6). These compounds play a key role in the bioactivity of citrus fruits, which are remarkable for their rich flavonoid content [14]. Thirteen flavonoid compounds were separated and identified using HPLC, and the results are displayed in Table (4). Ginger peels exhibited the highest content of flavonoid compounds (166.59 mg/100g) among each text plant compared to lemon peels, which recorded 74.22 mg/100g. Moreover, the predominant phenolic compounds were hesperidin for ginger peel (93.25 mg/100g) and hesperetin for lemon peels (18.65 mg/100g).

Ethanolic extract displayed the highest flavonoid content of all ginger and lemon peel extracts. Moreover, hesperidin was the major flavonoid compound in all plant extracts and lemon peels, which recorded 446.75 and 160.9 mg/100g for ethanolic and aqueous extracts of ginger peels and 116.39 and 95.17 mg/100g for ethanolic and aqueous extracts of lemon peels. On the other hand, the second flavonoid compound was kampferol 3-2-p-coumaroylglucose (154.71 and 111.85 mg/100g) for ethanolic and aqueous extracts of ginger peels. In comparison, it was naringin for ethanolic and aqueous extracts of lemon peels, which had 63.96 and 46.03 mg/100g, respectively.

It was also observed that the extraction process caused the elevation of some flavonoid compounds for ginger and lemon peel extracts, including rosmarinic, rutin, quercetrin, quercetin, and kampferol. Additionally, ethanolic extracts of ginger and lemon peels exhibited the highest flavonoid compounds (815.52 and 384.08 mg/100g) compared to aqueous extracts, which recorded 372.34 and 306.18 mg/100g, respectively. These results are in accordance with those reported by **Schieber *et al.*** [40] and **Sharma *et al.*** [41], who stated that the main flavonoids found in citrus fruits are hesperidin, narirutin, and naringin. As well as, the flavonoids compound contents of water extract for the (ginger: thyme: coriander) mixture using HPLC were Hesperidin Naringenin-7-oglucoside, Kaempferol 3.7 dirhamoside Luteolin 7 glucoside Naringenin, Hispertin and Apigenin (8599.6, 97.36, 228.16, 1448.9, 645.3, 109.3 and 29.9 mg/100g, respectively) [38]. The ethanol extracts exhibited significantly higher levels of flavonoid compounds compared to the aqueous extracts, as indicated in Table (1). This can be attributed to the higher polarity of ethanol, which enhances its extraction efficiency and enables the retrieval of a greater amount of active constituents and phytochemicals from the raw materials [39].

The antibacterial activity of ginger and lemon peel extracts

The widespread use of antibiotics often leads to the emergence of drug-resistant microbial strains. Because of this bacterial resistance, there is an immediate necessity to explore natural compounds possessing antibacterial properties [42]. Each plant extract was tested against four selected foodborne pathogenic bacteria, *Escherichia coli*, and *Salmonella typhimurium* as gram-negative for antibacterial activity. Moreover, *Staphylococcus aureus* and *Bacillus subtilis* as gram-positive bacteria at a concentration of 10 mg/mL compared to gentamycin as control at a concentration of 4 ug/ml, and the results are revealed in Table(5).

The ethanolic extract of both ginger and lemon peels demonstrated a higher degree of antibacterial activity than the aqueous extract. Furthermore, The highest antibacterial activity was observed in the ethanolic extract of ginger peels, with significant inhibition zones of 20.0 and 18.0 mm against gram-positive bacteria *Staph. aureus* and *B. subtilis*, and 17.0 and 15.0 mm against gram-negative bacteria *S. typhimurium* and *E. coli*. Following closely, the ethanolic extract of lemon peels exhibited inhibition zones of 16.0, 13.0, 15.0, and 12.0 mm, respectively. On the other hand, all studied strains were weakly inhibited by both aqueous extracts of ginger and lemon peels. Also, it was realized that the ethanolic extract of ginger peels was closer to the results of the antibiotic (Gentamycin). These results are in agreement with those of **Otang and Afolayan** [43], who mentioned that the antibacterial activity of lemon peel ethanolic extract was 15 mm against *E. coli*, 20 mm *S. typhimurium*, 17 mm *B. subtilis*, and 15 mm *Stap. Aureus*. Also, **Das *et al.*** [15] reported that the antibacterial activity of ginger peel ethanolic extract was 12 mm against *S. aureus* followed by *E. coli* (11 nm) and *Salmonella* spp. (8 nm).

Based on the obtained results, it can be inferred that the ethanolic extracts from ginger and lemon peels exhibit stronger antibacterial sensitivity against gram-positive bacteria than gram-negative bacteria. This observed difference is likely attributed to variations in the composition of their cell membranes. Notably, gram-negative bacteria feature an outer membrane containing lipopolysaccharides, rendering their cell walls impermeable to lipophilic substances. It contrasts with the cell structure of gram-positive bacteria, which lack this outer membrane. These morphological differences impact their antibacterial agent responsiveness[44].

Antifungal activity of ginger and lemon peel extracts

The antifungal activity of ethanolic and aqueous extracts of ginger and lemon peel was assayed against *Aspergillus flavus* and *Candida albicans*. The results are shown in Table (6). The ethanolic extract of ginger and lemon peels displayed the highest antifungal activity. The highest antifungal activity was observed against *C. albicans* (18.0 mm) and *A. flavus* (15.0 mm) for the ethanolic extract of ginger peel, followed by ethanolic extract of lemon peels, which recorded 15.0 and 12.0 mm, respectively for both strains. On the other hand, aqueous extract of lemon peel exhibited the lowest antifungal activity against both *A. flavus* and *C. albicans*. These results are similar to those of **Naqvi *et al.*** [33], who stated that ginger peels exhibited strong antifungal activity against *C. falcatum* and medium positive inhibition against *F. moniliforme*. Also, lemon peel extracts showed strong anti fungal activity against *C. albicans* and *Trichophyton rubrum*, ranging from 10.0 to 22.2 mm and 9.8 to 17.5 mm,

respectively [45] (Ali *et al.*, 2017). Also, It was observed that the ethanolic extract of ginger peels showed results more closely with the antibiotic (Ketoconazole).

Compared to the aqueous extract, the enhanced efficiency of the ethanolic extract against all tested foodborne pathogenic fungi can be attributed to ethanol's higher polarity. This greater polarity increases ethanol's capacity for extracting active constituents, such as phenolic and flavonoid compounds, contributing to its greater efficacy[46].

Cytotoxicity and anticancer activity of the ginger and lemon peel powder and their extracts on tumor cell lines
IC₅₀ (µg/ml)

The IC₅₀, which represents the concentration of the tested sample causing a 50% reduction in cell viability, was determined by adding serial dilutions of the ginger and lemon peel powder and their extracts to liver and colon carcinoma cells. The percentage of cells that survived at each concentration was determined, and the IC₅₀ value was determined graphically. The results are shown in Table (7) and Figure (3).

The data reveal that the ethanolic extract of ginger and lemon peels exhibited a pronounced cytotoxic effect and was found to possess very potent inhibitory activity against liver carcinoma cell line (HePG2) with an IC₅₀ value of 5.2 and 22.5 µg /ml, respectively, and colon carcinoma cell lines (HT-29) with IC₅₀ value of 4.7 and 19.5 µg /ml, respectively compared to aqueous extract of both raw materials. Meanwhile, the lowest anti-proliferative activity (IC₅₀) was found for ginger and lemon peel powder, which recorded 13.0 and 42.7 µg/ml against HT-29, while it recorded 31.7 and 8.7 µg/ml against HepG2, respectively. These results are in agreement with those of Zhang *et al.* [47] and El-Ashmawy *et al.*[47], who noted that ginger had potential anticancer effects against various cancer types, including breast, cervical, colorectal, and prostate cancer. Moreover, these results are lower than those of [48], who mentioned that the IC₅₀ value of lemon peel ethanolic extract was 97.8 µg/ml against HepG2 cells.

It was observed that gingerol and paradol, the most common compounds found in ginger extract, serve as potent antioxidants. They protect cells by neutralizing free radicals, harmful molecules that can generate chain reactions leading to cell damage. These compounds play a crucial role in enhancing the body's natural antioxidant defenses, making them valuable in preventing cell damage and inhibiting the growth of cancer cells [49]. As well as, lemon and their peels contain flavonoids like hesperetin, naringenin, quercetin, and diosmin. These compounds interact with genes and essential enzymes related to cell growth, the cell cycle, Apoptosis, and angiogenesis[50]. Notably, lemon powder and its extracts had the most anti-proliferative effects compared to ginger peel powder and its extracts. It could be attributed to the bioavailability of the active compounds in lemon peels, which may be

higher or more effective in inhibiting cancer cell growth than those in ginger peels. It could be due to the differences in how the body absorbs and utilizes the bioactive compounds present in these extracts[51][50].

Apoptotic and necrotic cells

Apoptotic cells undergo programmed cell death, a highly regulated and controlled process essential for maintaining tissue homeostasis and removing damaged cells. In contrast, necrotic cells experience uncontrolled, accidental cell death, typically resulting from physical injury, infection, or severe cellular stress. The key distinction lies in the orderly and organized nature of Apoptosis versus the chaotic and detrimental process of necrosis[52].

A one-way analysis of variance (ANOVA) demonstrates a highly significant distinction between the effects of ginger and lemon peel powder and their ethanolic extract on both apoptotic and necrotic cells. These findings are revealed in Table (9) and Figures (6 and 7). Among all samples, the ethanolic extract of ginger peel and its powder induced the highest level of cancer cell apoptosis against HT-29 cells (58.24 ± 0.13 and 45.62 ± 0.38 %, respectively) compared to lemon peel powder and its ethanolic extract, which displayed lower apoptotic values in tumor cells. Regarding necrotic cell results, ethanolic extract of ginger and lemon peels revealed the lowest percentage of necrotic cells, which displayed 1.96 ± 0.09 and 0.82 ± 0.38 %, respectively. These results are in agreement with those of **Karna et al.**[53] who reported that in mice treated with ginger extract, tumor microsections exhibited substantial tumor cell death, characterized by tumor necrosis, which turned to normal-looking healthy cells. Moreover, these results are in accordance with those of **Hamdan et al.** [54] and **Amorim et al.**[55] who mentioned that the anti-inflammatory activity of *C. limon* essential oil confirmed the interaction with TNF- α (tumor necrosis factor). Also, These results are similar to those **Zunino et al.** [56], who reported that limonin glucoside significantly decreased tumor necrosis factor - α (TNF- α) at the rate of (10.7%).

Ginger and lemon ethanolic extracts are rich in bioactive compounds known for their potential effects on apoptosis induction and necrosis reduction. Ginger, containing compounds like gingerol and paradol, exhibits anticancer properties and anti-inflammatory effects, suggesting a potential for apoptosis induction and necrosis reduction. The antioxidant properties of ginger may contribute to protecting cells from oxidative stress. Similarly, with flavonoids such as hesperidin, lemon showcases apoptotic potential and anti-inflammatory effects, supported by its antioxidant properties. Both extracts hold promise for influencing programmed cell death processes and minimizing uncontrolled cell death, but specific effects depend on factors like concentration and treatment duration. The connection between polyphenol content and the presence of apoptotic and necrotic cells could be attributed to polyphenol compounds that induce apoptotic cell death in cancer cells through various mechanisms. They act as antioxidants, cause cell cycle arrest, induce DNA damage, modulate apoptotic pathways, disrupt mitochondrial function, inhibit survival signals, and have anti-angiogenic effects[57][58][59].

Apoptotic/Necrotic ratio

The A/N Ratio, which assesses inflammatory potential, was used to compare ginger and lemon peel powder and their ethanolic extract. The analysis revealed a notable variance among the tested samples, as demonstrated in Table 9. Among ethanolic extracts of two materials, lemon had the highest ratio ($65.85 \pm 0.05\%$), while lemon peel powder showed the lowest ratio ($4.96 \pm 2.54\%$) among ginger and lemon powder. Notably, A/N Ratio > 1 : A higher ratio indicates a prevalence of apoptotic cell death over necrosis. This may suggest that the cells undergo a controlled physiological process or respond to a specific treatment that induces apoptosis [60]. Therefore, further research should aim to study the use of high concentrations of extracts, short treatment durations, and a narrow focus on apoptosis and necrosis mechanisms. Also, *in vivo* studies in animal models and, eventually, human clinical trials are needed to validate the anticancer potential and the cell death processes of these natural extracts fully.

Conclusion

Phenolic compounds and flavonoids, which are known to possess antioxidant activity and can be extracted to their maximum capacity using a hydroalcoholic solvent mixture, are commonly found in plant materials. The

phenolic compositions, antioxidant, antimicrobial, and anticancer properties of ginger and lemon peel powder and their extracts are simultaneously revealed in this study. The findings of the present research unequivocally demonstrate that lemon and ginger peels are excellent sources of phytonutrients with tremendous antioxidant capacity. In comparison to lemon peels, ethanol extracts of ginger peels are more effective at extracting phenolic, flavonoid, antioxidant, and antimicrobial compounds. Furthermore, it has been observed that ethanolic extracts, specifically those derived from ginger peel, exhibit significantly stronger cytotoxic effects on liver and colon carcinoma cells in comparison to lemon peel. Analyses of apoptotic and necrotic cells demonstrate that ginger peel inhibits necrosis while inducing apoptosis in cancer cells. As a result, ethanolic extracts of ginger and lemon peels have considerable potential as natural alternatives to conventional treatments for chronic diseases like cancer and bacterial/fungal infections.

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Disclosure

There are no conflicts of interest in this work

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