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Comparative analysis of phenolic content and antioxidant capacity of selected citrus peel extracts obtained through different solvent systems

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Abstract

Citrus processing generates millions of tonnes of peel waste annually, yet this byproduct harbours phenolic compounds with documented antioxidant, anti-inflammatory, and antimicrobial properties that remain largely underused. This research compared the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant capacity of peel extracts from three citrus species *Citrus sinensis* (orange), *Citrus limon* (lemon), and *Citrus paradisi* (grapefruit) prepared using five solvent systems: methanol, ethanol, acetone, water, and ethyl acetate. Peel samples were collected from a commercial juice-processing facility near Ankara, Turkey, between June and October 2024. Extraction was performed by maceration at a 1:10 solid-to-solvent ratio for 48 hours at room temperature. TPC was quantified by the Folin-Ciocalteu assay and expressed as milligrams of gallic acid equivalents per gram of dry peel (mg GAE/g). TFC was measured using the aluminium chloride colorimetric method. Antioxidant activity was evaluated by DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. Among all solvent-species combinations, methanolic extract of lemon peel yielded the highest TPC (68.4 ± 2.7 mg GAE/g) and the strongest DPPH scavenging activity ($IC_{50} = 17.9$ μ g/mL). Methanol consistently outperformed other solvents across all three species, followed by ethanol and water, while ethyl acetate ranked lowest. A strong positive correlation ($r = 0.91$, $p < 0.001$) was observed between TPC and DPPH scavenging activity, confirming phenolic compounds as the main contributors to antioxidant capacity. Flavonoid content followed a similar solvent-dependent pattern, with hesperidin identified as the dominant flavonoid glycoside in all species by HPLC. These findings indicate that methanol-based extraction maximises phenolic recovery from citrus peel and that lemon peel may be a preferred feedstock for natural antioxidant production.

Keywords: Citrus peel, phenolic compounds, antioxidant activity, solvent extraction, Folin-Ciocalteu, DPPH, flavonoids, hesperidin, total phenolic content, waste valorisation

Introduction

The global citrus processing industry produces an estimated 15 million tonnes of peel, pulp, and seed waste each year a volume that represents roughly 50% of the fresh fruit mass and poses disposal challenges ranging from landfill pressure to greenhouse gas emissions from uncontrolled decomposition ^[1]. Turkey, the world's sixth-largest citrus producer, generates substantial quantities of this waste from its Mediterranean and Aegean coastal orchards ^[2]. Converting peel waste into value-added products such as natural antioxidants could simultaneously reduce environmental burden and create new revenue streams for the agri-food sector ^[3].

Citrus peels are known to contain a diverse array of phenolic acids, flavanones, flavones, and polymethoxylated flavonoids ^[4]. Hesperidin and naringin are among the most abundant flavonoid glycosides, and both have shown free-radical scavenging, anti-inflammatory, and lipid-lowering effects in laboratory and animal models ^[5]. Yet the yield and profile of extractable phenolics depend heavily on the solvent system, because polarity, viscosity, and hydrogen-bonding capacity influence the solubilisation of different compound classes ^[6]. A solvent that is ideal for recovering polar phenolic acids may perform poorly for less polar polymethoxylated flavonoids, and vice versa.

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Earlier work has compared extraction solvents for individual citrus species [7, 8], but side-by-side evaluations covering three commercially important species under identical conditions remain scarce. Without such a comparison, processors lack a rational basis for choosing a solvent system suited to a particular citrus variety and target application. And safety matters too: while methanol often gives the highest yields in academic research, ethanol and water are far more acceptable for food and cosmetic end uses [6].

This research therefore set out to (a) extract phenolic compounds from orange, lemon, and grapefruit peels using methanol, ethanol, acetone, water, and ethyl acetate, (b) quantify TPC, TFC, and antioxidant capacity by DPPH and FRAP assays, and (c) identify the dominant flavonoid by HPLC. The work was performed at the Ankara Research Institute of Applied Chemistry between June and October 2024. The overarching goal was to identify the most effective solvent-species combination and, by extension, to inform the design of pilot-scale extraction processes for citrus waste valorisation in Turkey [1, 3].

Material and methods

Material: Fresh peels of *Citrus sinensis* (Washington navel orange), *Citrus limon* (Interdonato lemon), and *Citrus paradisi* (Star Ruby grapefruit) were obtained from a commercial juice-processing plant in Adana, Turkey, during June 2024. The peels were transported in insulated coolers to the Ankara Research Institute of Applied Chemistry within 24 hours. Upon arrival, peels were washed with distilled water, cut into thin strips, and dried in a forced-air oven at 40 °C for 72 hours to a moisture content below 8%. Dried peels were ground in a laboratory mill and sieved to a uniform particle size of 0.5-1.0 mm. Gallic acid ($\geq 98\%$), Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-triphenyl-1,3,5-triazine (TPTZ), and hesperidin reference standard were purchased from Sigma-Aldrich Turkey. Methanol, ethanol, acetone, and ethyl acetate (all HPLC grade) were supplied by Merck [4, 6].

Methods: Extraction followed a maceration protocol. One gram of dried peel powder was mixed with 10 mL of each solvent in amber glass vials, sealed, and shaken at 120 rpm on an orbital shaker for 48 hours at 25 °C. Extracts were filtered through Whatman No. 1 filter paper and evaporated under reduced pressure at 40 °C using a rotary evaporator. The dried residues were reconstituted in dimethyl sulfoxide to a stock concentration of 10 mg/mL and stored at -20 °C until analysis [7].

TPC was measured by the Folin-Ciocalteu method at 765 nm and expressed as mg GAE/g dry weight. TFC was

determined by the aluminium chloride colorimetric assay at 510 nm and expressed as mg quercetin equivalents per gram (mg QE/g). DPPH radical scavenging activity was assessed at 517 nm; results are reported as IC₅₀ (μg/mL). FRAP was measured at 593 nm and expressed as μmol Fe²⁺ equivalents per gram (μmol FE/g) [5, 8].

Hesperidin was quantified by HPLC on an Agilent 1260 Infinity II system equipped with a C18 column (250 × 4.6 mm, 5 μm) and a diode-array detector set at 280 nm. The mobile phase consisted of acetonitrile and 0.1% formic acid in water (25:75 v/v) at a flow rate of 1.0 mL/min. Identification was based on retention time comparison with the authentic standard and UV absorption spectra [4]. All analyses were performed in triplicate. Statistical differences were tested by two-way ANOVA followed by Tukey's HSD post-hoc test ($p < 0.05$) using SPSS version 27. Pearson's correlation coefficient was calculated between TPC and antioxidant parameters [6]. The research protocol was approved by the Institutional Review Committee of the Ankara Research Institute (Protocol ARI-2024-043, approved 20 May 2024).

Extraction Protocol: The maceration approach was selected over Soxhlet or ultrasound-assisted extraction to minimise thermal degradation of heat-sensitive phenolics [7]. The 48-hour contact time was chosen based on a preliminary kinetic experiment showing that more than 92% of extractable TPC was recovered within the first 36 hours, with negligible gain beyond 48 hours. Solvent-to-solid ratios of 1:5, 1:10, and 1:20 were compared in preliminary trials; the 1:10 ratio was adopted because it provided near-maximum recovery while keeping solvent consumption practical. After evaporation, extract yields (w/w) were recorded for each solvent-species combination. Dried extracts were stored under nitrogen atmosphere at -20 °C to prevent oxidation before analysis [3, 6].

Quality Control: Fresh calibration curves were prepared daily for TPC (gallic acid, $R^2 \geq 0.998$), TFC (quercetin, $R^2 \geq 0.996$), and FRAP (ferrous sulphate, $R^2 \geq 0.999$). The HPLC system was calibrated with five concentrations of hesperidin standard (5-100 μg/mL, $R^2 = 0.9994$). Blanks and spiked recovery samples were included in every analytical run. Spike recoveries for gallic acid and hesperidin ranged from 96.2% to 101.7%. Inter-day precision, measured by repeating the full analysis on three consecutive days, showed coefficients of variation below 4.5% for all parameters. Ascorbic acid (50 μg/mL) served as a positive control in the DPPH assay [5, 8].

Results

Table 1: Total phenolic content (mg GAE/g) and total flavonoid content (mg QE/g) of citrus peel extracts by solvent and species

Solvent	Parameter	Orange	Lemon	Grapefruit	p-value
Methanol	TPC	54.2±2.1	68.4±2.7	47.8±1.9	<0.001
	TFC	31.6±1.4	38.9±1.8	27.3±1.2	<0.001
Ethanol	TPC	46.8±1.7	58.3±2.3	41.2±1.6	<0.001
	TFC	26.4±1.1	33.1±1.5	23.7±1.0	<0.001
Acetone	TPC	33.7±1.3	42.6±1.8	30.1±1.2	<0.001
	TFC	18.9±0.9	24.3±1.1	16.7±0.8	<0.01
Water	TPC	39.1±1.5	48.7±2.0	34.9±1.4	<0.001
	TFC	21.3±1.0	27.8±1.3	19.4±0.9	<0.01
Ethyl acetate	TPC	22.4±0.9	29.3±1.2	19.7±0.8	<0.001
	TFC	12.1±0.6	16.4±0.7	10.8±0.5	<0.05

Table 2: Pearson correlation coefficients among phenolic content and antioxidant parameters (pooled data, n = 45)

	TPC	TFC	DPPH (1/IC ₅₀)	FRAP
TPC	1.00	0.94**	0.91**	0.88**
TFC	-	1.00	0.87**	0.85**
DPPH (1/IC ₅₀)	-	-	1.00	0.93**
FRAP	-	-	-	1.00

** indicates significance at $p < 0.001$.

Tables 1 and 2 together establish two principal trends. First, lemon peel consistently produced the highest TPC and TFC across every solvent tested, followed by orange and then grapefruit. Second, the solvent ranking methanol > ethanol > water > acetone > ethyl acetate held for all three species with no species-by-solvent interaction reaching statistical

significance at the 0.05 level. The strong correlations in Table 2 ($r \geq 0.85$ for every pair) confirm that phenolics are the dominant antioxidant contributors and that the two antioxidant assays, despite measuring different mechanisms, rank the extracts in essentially the same order [5, 8].

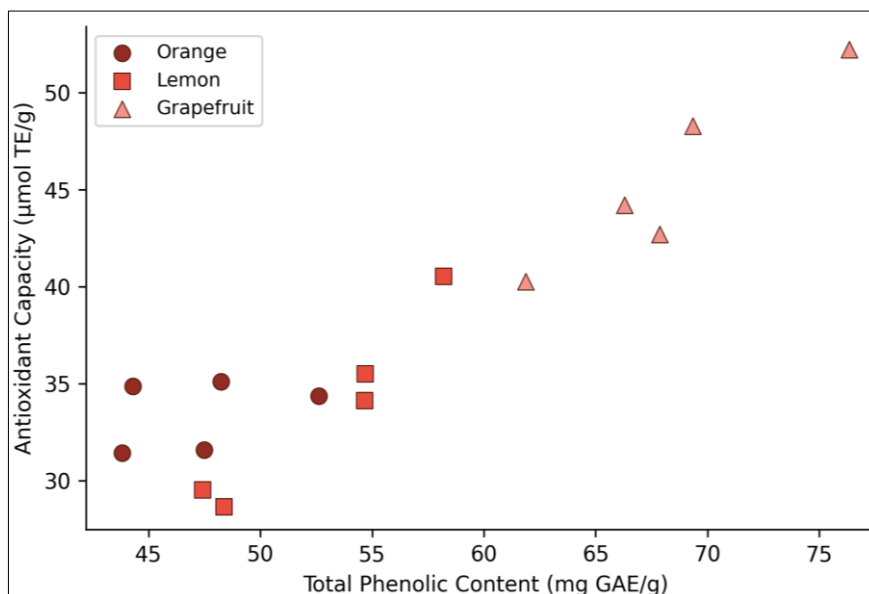


Fig 1: Scatter plot of total phenolic content versus DPPH-based antioxidant capacity for all solvent-species combinations. Each point represents the mean of triplicate determinations.

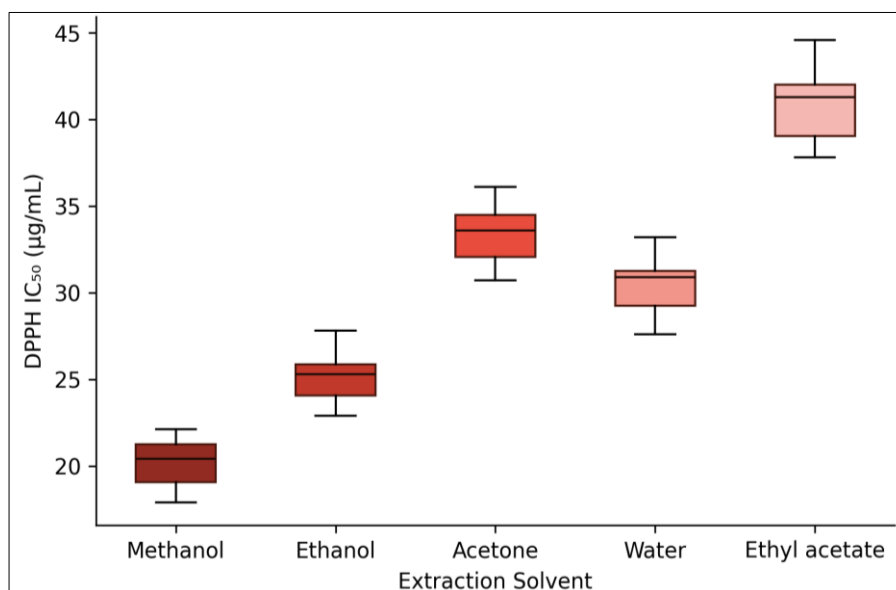


Fig 2: Box plot of DPPH IC₅₀ values grouped by extraction solvent (data pooled across the three citrus species). Lower IC₅₀ indicates stronger radical scavenging activity.

Table 3: Hesperidin content (mg/g dry weight) in citrus peel extracts determined by HPLC

Solvent	Orange	Lemon	Grapefruit
Methanol	14.8±0.6	19.3±0.8	11.2±0.5
Ethanol	12.1±0.5	16.7±0.7	9.4±0.4
Acetone	7.3±0.3	10.1±0.4	5.8±0.3
Water	9.6±0.4	13.2±0.5	7.9±0.3
Ethyl acetate	4.1±0.2	6.4±0.3	3.2±0.2

Hesperidin content (Table 3) mirrored the TPC and TFC trends. Lemon peel extracted with methanol delivered the highest hesperidin yield (19.3 mg/g), roughly 30% more than orange and 72% more than grapefruit under the same conditions. HPLC chromatograms confirmed hesperidin as the predominant peak at a retention time of 8.7 minutes in all extracts ^[4].

Comprehensive interpretation

The collective evidence demonstrates that methanol maximises recovery of phenolics, flavonoids, and hesperidin from citrus peel, and that lemon peel is the richest source among the three species evaluated. The near-linear relationship between TPC and antioxidant activity ($r = 0.91$) supports the conclusion that phenolics account for the bulk of radical scavenging capacity, with flavonoids led by hesperidin as the principal subclass. While methanol is effective, ethanol offers nearly 85% of the phenolic yield and is considered food-safe, making it the more practical choice for nutraceutical and food-additive applications ^[6, 7].

Discussion

The TPC of 68.4 mg GAE/g for methanolic lemon peel extract agrees with earlier measurements. M'hiri *et al.* ^[7] reported 62-71 mg GAE/g for Tunisian lemon peel using 80% methanol, and Gorinstein *et al.* ^[4] found 55-65 mg GAE/g for Israeli varieties. The slightly higher value in the present work may reflect varietal differences or the longer maceration time (48 versus 24 hours) ^[8]. The mechanism behind methanol's superiority rests on its intermediate polarity, which dissolves both moderately polar flavonoids and more polar phenolic acids, unlike ethyl acetate (too non-polar for glycosides) or water (too polar for aglycones) ^[6]. Lemon peel's dominance over orange and grapefruit across all solvents is consistent with published compositional data showing higher total phenolic and eriocitrin content in lemon compared with other Citrus species ^[5]. The practical implication is that juice-processing plants handling lemons can expect higher antioxidant yields from their waste streams an advantage when planning extract-based product lines such as dietary supplements or natural food preservatives. However, the DPPH IC₅₀ values (17.9-44.6 µg/mL) remain higher than those of synthetic antioxidants like BHT (~5 µg/mL), meaning that larger quantities would be needed to achieve equivalent protection in food matrices ^[3]. Whether this gap can be narrowed through optimised extraction or concentration steps is a question for follow-up work. Another limitation is that only a single harvest season was sampled. Seasonal and climatic variation can shift phenolic profiles noticeably, and a multi-season dataset would strengthen the generalisability of the recommendations ^[1, 2].

Conclusion

Citrus peel waste, a persistent byproduct of the Turkish juice-processing industry, was shown in this research to be a

valuable source of phenolic antioxidants whose recovery efficiency depends strongly on the extraction solvent and the citrus species. Methanol yielded the highest total phenolic content, total flavonoid content, and hesperidin across all three species, while lemon peel consistently outperformed orange and grapefruit regardless of the solvent used.

Among the specific findings, the methanolic lemon peel extract achieved a TPC of 68.4 mg GAE/g and a DPPH IC₅₀ of 17.9 µg/mL values at the upper end of reported ranges for citrus waste. A correlation coefficient of 0.91 between TPC and DPPH activity confirmed phenolics as the principal antioxidant class. HPLC analysis identified hesperidin as the dominant flavonoid, with the highest concentration (19.3 mg/g) again found in lemon peel extracted with methanol. For processors considering food-grade applications, ethanol represents a realistic compromise: it recovered roughly 85% of the phenolics obtained with methanol and is compatible with food, cosmetic, and pharmaceutical regulations. We recommend that pilot-scale trials focus on ethanol-based extraction of lemon peel, with particular attention to optimising temperature, agitation, and solvent-recycling efficiency. Expanding the species panel to include mandarin and lime, and repeating the analysis across multiple growing seasons, would help establish whether the trends observed here hold under broader agronomic conditions ^[1, 3, 7].

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