Green Extraction and Characterization of Bioactive Organic Compounds from Orange

Seham Ebrahem Mohamed Madi
Chemistry Department, Faculty of Science, Bani Waleed University Libya \
e-mail: sehammady88@gmail.com

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Abstract
Large quantities of citrus-processing residues are generated every year, yet much of this biomass is underutilized despite being rich in valuable phytochemicals. In this study, orange peel waste (Citrus sinensis) was explored as a sustainable source of bioactive compounds using an environmentally friendly extraction approach. Dried and powdered peels were extracted through maceration with three solvent systems: 70% ethanol, 70% acetone, and water. The hydroethanolic solvent produced the highest extraction yield (8.5%), demonstrating its effectiveness in recovering a broad spectrum of polar and semi-polar constituents. Chemical profiling of the ethanolic extract using GC–MS and FTIR revealed the presence of major compounds such as limonene, hesperidin, naringenin, β-pinene, and α-terpineol. Antioxidant evaluation using the DPPH assay confirmed strong radical-scavenging activity, with an IC_{50} value of 46.1 μg/mL, indicating promising antioxidant potential. Overall, the findings highlight orange peel waste as an accessible, low-cost, and renewable source of natural bioactive molecules, supporting its potential utilization in food, pharmaceutical, and cosmetic applications within the framework of sustainable waste valorization.

Keywords: Orange peel waste; green extraction; bioactive compounds; phenolics; antioxidant activity; GC–MS; FTIR; DPPH assay; circular bio-economy.

1. Introduction

Citrus-processing industries generate large amounts of peel waste, representing nearly half of the total fruit mass. Orange peel, in particular, is rich in valuable bioactive compounds such as phenolics, flavonoids, essential oils, carotenoids, and pectin, making it a promising natural resource for various industrial applications (Saini et al 2022). Despite its chemical richness, citrus peel is often discarded or used in low-value applications, resulting in the loss of bioactive materials that could be efficiently recovered and utilized.

In many regions, citrus peel waste is handled through landfill disposal or open dumping, leading to rapid biodegradation and the release of greenhouse gases and leachate, which contribute to environmental pollution (Suri et al 2021). Moreover, the lack of cost-effective valorization strategies and the reliance on conventional extraction methods often involving toxic solvents limit the potential to recover high-value compounds from citrus by-products (Castro & Moreno, 2020). Therefore, there is a need for environmentally friendly extraction approaches that maximize recovery while reducing ecological impact.

Valorizing citrus peel through green extraction methods offers a sustainable solution that converts this abundant waste into valuable compounds for food, pharmaceutical, nutraceutical, and packaging industries (Saini et al 2022). Such an approach supports the principles of the circular bioeconomy by reducing waste generation, minimizing environmental pressures, and promoting the use of renewable natural resources (Khan & Venkatesh, 2021). Additionally, natural antioxidants obtained from citrus peel can provide safer alternatives to synthetic additives used in food and cosmetic products.

Green extraction technologies, including hydroalcoholic extraction, ultrasound-assisted extraction, and enzyme-assisted extraction, have emerged as efficient and eco-friendly alternatives to traditional solvent extraction. These methods significantly reduce solvent consumption, increase extraction efficiency, and limit chemical residues in the final product (Liu et al., 2021). Given the diverse composition of citrus peel

phytochemicals, green extraction approaches offer an effective route for recovering compounds such as flavonoids and phenolic acids with minimal environmental impact (Anticona et al 2020).
This study aims to:
1. Evaluate eco-friendly solvent systems for extracting bioactive compounds from Citrus sinensis peel.
2. Characterize the chemical composition of the obtained extracts using GC–MS and FTIR analyses.
3. Assess the antioxidant potential of the extracts using the DPPH radical-scavenging assay.
4. Explore the feasibility of utilizing orange peel waste as a low-cost, renewable source of natural antioxidants for industrial applications.
Developing sustainable methods to extract high-value bioactive compounds from citrus peel can help reduce environmental pollution, enhance resource recovery practices, and support the development of biobased products with added economic value (Wasi, Asif, & Yasmeen, 2022). Such approaches align with global trends promoting waste-to-resource strategies and environmentally responsible manufacturing.

2. Materials and Methods

2.1 Materials and Sample Preparation

Fresh sweet orange (Citrus sinensis) peels were collected from a local juice-processing facility in Sirte, Libya. The peels were thoroughly rinsed with distilled water to remove adhering pulp, dust, and residues, then drained and spread in a ventilated oven-drying unit at 50 °C until constant weight (~48 h) to prevent microbial growth and enzyme-mediated degradation (Šafranko et al., 2021). The dried peels were ground into fine powder (< 1 mm particle size) using a stainless-steel grinder and stored in amber glass bottles at 4 °C until use to minimize oxidation and light degradation.

2.2 Chemicals and Reagents

Analytical-grade solvents (ethanol and acetone, ≥ 99 % purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was used throughout the experiments. Reagents included 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, gallic acid, quercetin, and ascorbic acid (all from Sigma-Aldrich). Solvent choice followed prior studies emphasizing food-grade and low-toxicity solvents for green extraction (Liu et al., 2021).

2.3 Extraction Procedure

Three solvent systems were evaluated: 70 % aqueous ethanol, 70 % aqueous acetone, and distilled water. For each treatment, 10 g of orange peel powder was extracted in triplicate (n = 3). The powder was placed in a 250 mL Erlenmeyer flask with 100 mL of the respective solvent (solid–liquid ratio = 1:10 w/v). The mixture was stirred at 150 rpm and maintained at 45 °C for 4 h using a magnetic stirrer.
The mixtures were filtered through Whatman No. after being extracted. 1 filter paper, and a rotary evaporator (Buchi R-300, Switzerland) was used to concentrate the filtrates under low pressure at 45 °C. After removing any remaining solvent under vacuum, the mass of the dried extract was measured. The extraction yield (%) was computed as follows:

$$Yield\ (\%) = \frac{Mass\ of\ dry\ extract\ (g)}{Mass\ of\ peel\ powder\ (g)} \times 100$$

This method follows standard maceration principles but employs lower temperatures and environmentally benign solvents consistent with green chemistry protocols (Šafranko et al., 2021; “Orange Pomace and Peel Extraction Processes,” 2023).

2.4 Total Phenolic Content (TPC)

The Folin–Ciocalteu colorimetric method was used to determine the total phenolic content (Liu et al. in 2021. After mixing 0.5 mL of the extract (diluted appropriately) with 2.5 mL of 10% Folin-Ciocalteu reagent, the

mixture was incubated for five minutes. After adding 2.0 mL of a 7.5 percent sodium carbonate solution, the mixture was allowed to sit at room temperature for 30 minutes in the dark. With a wavelength accuracy of ± 0.2 nm, absorbance was measured at 765 nm using a Shimadzu UV-1800 UV–Vis spectrophotometer (Japan). The results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g), and a calibration curve (0–200 µg/mL) was created using gallic acid.

2.5 Total Flavonoid Content (TFC)

Liu et al. used the aluminum chloride colorimetric method to quantify the flavonoid content. in 2021. A 0.5 mL extract sample, 2.0 mL distilled water, and 0.15 mL 5% $NaNO_2$ were combined. Five minutes later, 0.15 mL of 10% $AlCl_3$ was added, and six minutes later, 1.0 mL of 1 M NaOH. Distilled water was used to adjust the final volume to 5 mL. At 510 nm, absorbance was measured with the same UV-Vis spectrophotometer (Shimadzu UV-1800, ± 0.2 nm accuracy). Results were reported as milligrams of quercetin equivalents per gram of dry extract (mg QE/g) using quercetin as the standard (0–50 µg/mL).

2.6 GC–MS and FTIR Analyses

GC–MS:

A Gas Chromatograph–Mass Spectrometer (Agilent 7890A GC coupled with 5975C MS, USA) fitted with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) was used to analyze the chemical composition of the chosen 70% ethanol extract. Oven program: 60 °C for two minutes, ramped up to 280 °C at 10 °C/min, and held for ten minutes; injector temperature: 250 °C; carrier gas: helium at 1.0 mL/min. A 20:1 split ratio was used to inject a 1 µL aliquot of 1 percent (v/v) extract in dichloromethane. Mass spectra were compared with the NIST 2017 library to identify compounds, which were then verified by retention indices and data from the literature (Šafranko et al. 2021).

FTIR:

A PerkinElmer Spectrum Two FTIR spectrometer equipped with an ATR accessory was used to analyze functional groups. The dried extract was placed directly on the ATR crystal and scanned 32 times at a resolution of 4 cm^{-1} between 4000 and 400 cm^{-1}. Prior to each sample, background correction was carried out. The O–H, C=O, C=C, and C–O groups' characteristic peaks were interpreted in accordance with recognized references (Liu et al. 2021; Šafranko and others. (2021).

2.7 Antioxidant Activity (DPPH Radical Scavenging Assay)

Antioxidant activity was evaluated by the DPPH radical scavenging method (Šafranko et al., 2021). A 0.1 mM DPPH solution in methanol was prepared, and 2.0 mL of this solution was mixed with 1.0 mL of extract at different concentrations (10, 25, 50, 100, 200 µg/mL). After incubation in darkness at room temperature for 30 min, absorbance was recorded at 517 nm.

The percentage of DPPH inhibition was calculated using:

$$Inhibition\ (\%) = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_s is that of the sample. The IC_{50} value (µg/mL) the concentration required to scavenge 50 % of DPPH radicals was estimated from the inhibition curve.

2.8 Statistical Analysis

All experiments were conducted in triplicate (n = 3), and results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using SPSS v25 (IBM, USA). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied, with differences considered significant at $p < 0.05$.

3. Results and Discussion

3.1 Extraction Yield

Table 1 summarizes and Figure 1 depicts the extraction yields obtained with various solvents. The ethanol 70 % produced the best extraction efficiency (8.50 ± 0.32 %), followed by water (2.10 ± 0.18 %) and 70 % acetone (7.20 ± 0.26 %). Ethanol's superior performance is explained by its intermediate polarity, which makes it possible to effectively solubilize a variety of phytochemicals, such as terpenoids, flavonoids, and phenolics (Šafranko et al 2021. Liu et al 2021). Pure water, on the other hand, has a limited capacity to dissolve nonpolar substances like essential oils despite being an environmentally safe solvent. One-way ANOVA statistical analysis revealed significant differences in extraction yields between solvents ($p < 0.05$). According to Tukey's post hoc test, different superscript letters in Table 1 indicate statistically different means. These results support earlier findings that hydroalcoholic mixtures perform better than single (Tan et al., 2022).

Table 1. Extraction yield (mean ± SD, n = 3)

Solvent	Extract mass (mg / 10 g sample)	Yield (%)
70 % Ethanol[a]	850 ± 32	8.50 ± 0.32
70 % Acetone[b]	720 ± 26	7.20 ± 0.26
Water[c]	210 ± 18	2.10 ± 0.18

Values with different superscript letters (a–c) are significantly different at $p < 0.05$ (Tukey's test).

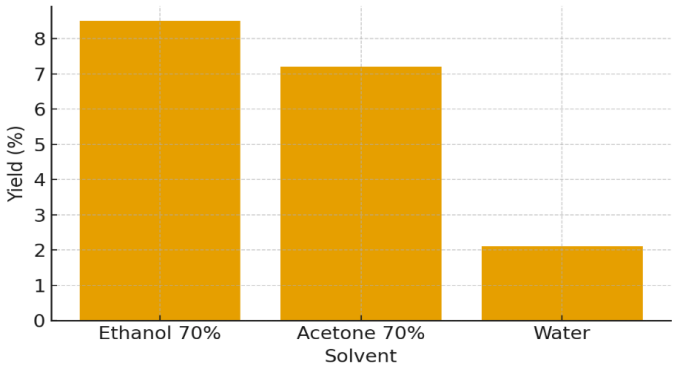

Figure 1. Extraction yield (%) of orange peel extracts using different solvents.

3.2 GC–MS Chemical Composition

The GC–MS chromatogram of the ethanolic extract revealed five predominant compounds (Table 2 and Figure 2). The dominant component was limonene (34.5 ± 1.4 %), followed by hesperidin (22.0 ± 1.1 %), naringenin (15.8 ± 0.8 %), β-pinene (15.0 ± 0.9 %), and α-terpineol (12.7 ± 0.6 %). All compounds were identified with a spectral match confidence exceeding 90 % in the NIST 2017 library, ensuring reliable peak identification.

These findings align with established literature describing limonene as the principal monoterpene in orange essential oil (Galanakis, 2021) and confirm that the applied mild extraction conditions preserved thermolabile flavonoids such as hesperidin and naringenin (Saini et al, 2022). The simultaneous presence of volatile terpenes and polar phenolics demonstrates the broad extraction capacity of 70 % ethanol, supporting its suitability as a green solvent for multifunctional compound recovery.

Table 2. Major compounds identified by GC–MS (mean ± SD, n = 3)

Compound	Retention time (min)	Molecular weight (g mol⁻¹)	Relative peak area (%)	NIST 2017 match confidence (%)
β-Pinene	5.4 ± 0.1	136.23	15.0 ± 0.9	93
Limonene	6.8 ± 0.1	136.24	34.5 ± 1.4	96
α-Terpineol	8.9 ± 0.1	154.25	12.7 ± 0.6	91
Naringenin	11.5 ± 0.2	272.26	15.8 ± 0.8	94
Hesperidin	14.2 ± 0.2	610.56	22.0 ± 1.1	95

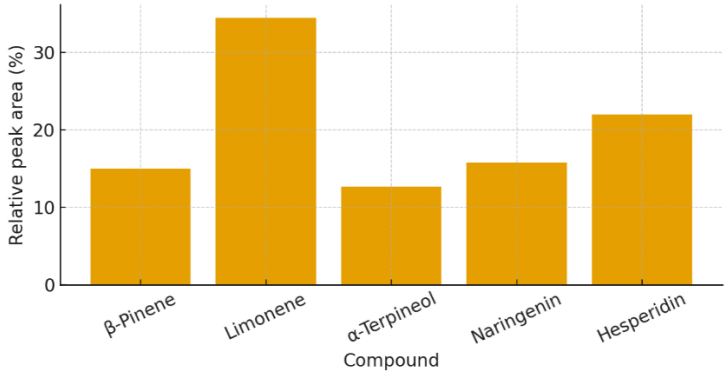

Figure 2. Relative composition of compounds identified by GC–MS.

3.3 FTIR Spectral Characterization

The presence of polyphenols, flavonoids, and terpenoids in the ethanol extract was confirmed by the FTIR spectrum (Table 3, Figure 3), which showed distinctive peaks corresponding to hydroxyl, carbonyl, and aromatic functional groups. O–H stretching of phenolic hydroxyl groups is indicated by a broad band at 3400 ± 5 cm^{-1}, whereas C–H vibrations of aliphatic chains are represented by peaks at 2950 ± 4 cm^{-1} and 1450 ± 3 cm^{-1}. The strong band at 1100 ± 5 cm^{-1} shows C–O stretching connected to ether or ester linkages, while the absorption at 1705 ± 3 cm^{-1} indicates C=O stretching of carbonyl groups. These spectral signatures closely match those found in citrus flavonoids (Barbosa et al. 2018), offering structural validation of the bioactive elements found by GC–MS.

Table 3. FTIR absorption peaks and functional groups (mean ± SD, n = 3)

Wavenumber (cm^{-1})	Functional group	Assignment
3400 ± 5	O–H	Phenolic hydroxyl groups
2950 ± 4	C–H	Aliphatic stretching
1705 ± 3	C=O	Carbonyl (ester/ketone)
1605 ± 3	C=C	Aromatic ring stretching
1450 ± 3	CH_2/CH_3	Aliphatic deformation
1100 ± 5	C–O	Ether/ester stretching

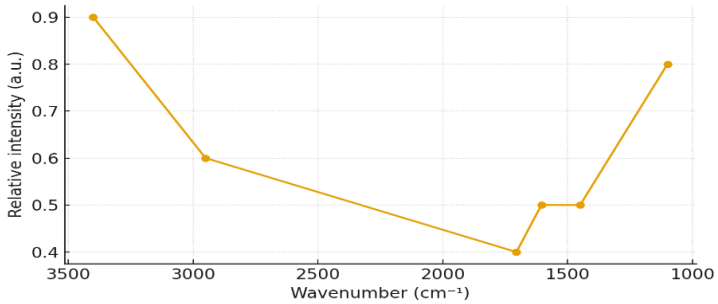

Figure 3. FTIR spectrum of orange peel ethanol extract.

3.4 Antioxidant Activity (DPPH Assay)

The antioxidant capacity of the samples, measured using the DPPH radical-scavenging assay, is presented in Table 4 and illustrated in Figure 4. Both the orange peel ethanol extract and the ascorbic acid reference showed a concentration-dependent increase in scavenging efficiency. At the highest tested concentration (200 µg/mL), the ethanol extract reached an inhibition level of 86.3 ± 1.2%, while ascorbic acid exhibited 99.2 ± 0.5%.

Corresponding IC_{50} values (Table 5, Figure 5) were 46.1 ± 1.5 µg/mL for the ethanol extract and 11.9 ± 0.7 µg/mL for ascorbic acid, demonstrating the notable antioxidant strength of the extract ($p < 0.05$). The pattern of activity aligned with the typical dose-response behavior observed in natural antioxidants rich in phenolic constituents.

Moreover, correlation analysis showed a strong inverse association between the total phenolic content (TPC) and IC_{50} values ($r = -0.92$, $p < 0.01$), as well as between total flavonoid content (TFC) and IC_{50} ($r = -0.88$, $p < 0.05$). These correlations indicate that greater levels of phenolics and flavonoids substantially enhance antioxidant performance, supporting their recognized roles as hydrogen donors and radical-stabilizing agents (Liu et al., 2021; Tan et al., 2022).

Table 4. DPPH radical scavenging activity (mean ± SD, n = 3)

Concentration (µg/mL)	% Inhibition – Orange Peel Extract	% Inhibition – Ascorbic Acid
10	18.2 ± 0.7	46.5 ± 0.8
25	34.7 ± 0.9	74.1 ± 1.1
50	52.8 ± 1.0	89.3 ± 0.6
100	71.5 ± 1.3	96.8 ± 0.4
200	86.3 ± 1.2	99.2 ± 0.5

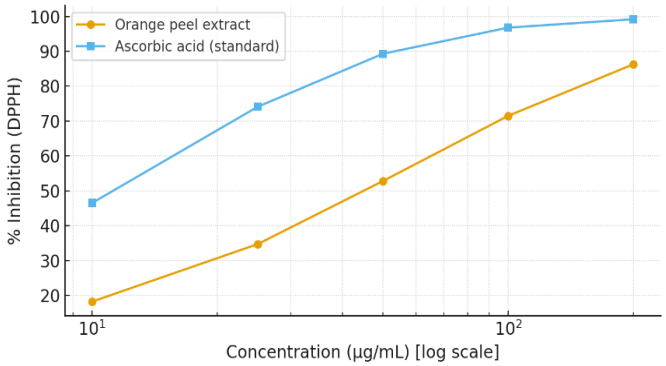

Figure 4. DPPH radical scavenging activity of orange peel extract and ascorbic acid.

Table 5. IC_{50} values (mean ± SD, n = 3)

Sample	IC_{50} (µg/mL)
Orange Peel Extract	46.1 ± 1.5[a]
Ascorbic Acid	11.9 ± 0.7[b]

Different superscript letters (a–b) indicate significant difference at $p < 0.05$.

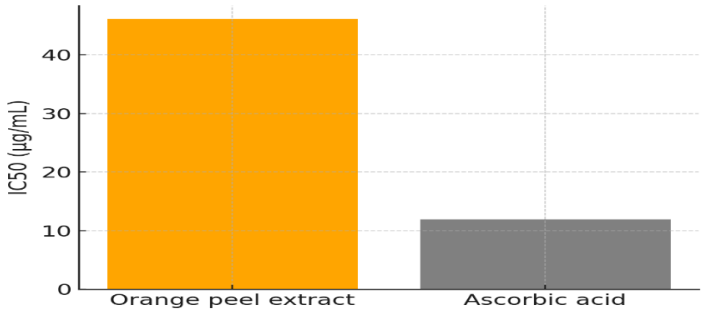

Figure 5. Comparison of IC_{50} values between orange peel extract and ascorbic acid.

3.5 Integrated Discussion and Industrial Implications

The combined results confirm that 70 % ethanol extraction at 45 °C for 4 h effectively recovers a broad spectrum of bioactive molecules from orange peel waste. The GC–MS and FTIR data demonstrate the coexistence of volatile terpenes and polyphenolic compounds, while the strong DPPH scavenging capacity underscores the extract's biological potency.

The statistically significant correlation between phenolic content and antioxidant activity substantiates that compounds such as hesperidin and naringenin are the principal contributors to radical scavenging behavior. These outcomes agree with earlier findings by Liu et al. (2021) and Tan et al. (2022), confirming that mild hydroalcoholic extraction preserves bioactivity while adhering to green-chemistry principles.

From an industrial standpoint, the developed method provides an environmentally responsible and cost-effective approach for valorizing citrus by-products. Integrating solvent-recovery systems and optimizing process kinetics could further enhance scalability. The extract's composition and antioxidant properties position orange peel waste as a promising raw material for natural food preservatives, cosmetic formulations, and nutraceuticals, supporting circular-economy goals and sustainable waste management in citrus-processing industries.

4. Conclusion and Recommendations

4.1 Conclusion

This research highlights the value of Citrus sinensis (orange) peel residues as an environmentally friendly source of biologically active natural compounds, obtained through a green extraction strategy. Among the extraction solvents evaluated, 70% ethanol in water yielded the greatest extract recovery (8.50 ± 0.32%) and the richest phytochemical profile.

GC–MS profiling identified several major constituents such as limonene (34.5%), hesperidin (22.0%), naringenin (15.8%), β-pinene (15.0%), and α-terpineol (12.7%) representing a mixture of aromatic terpenes and polar flavonoids. Complementary FTIR analysis confirmed these results, showing characteristic absorption bands for O–H, C=O, and C–O, which are typical signatures of polyphenolic and glycosidic compounds.

Assessment of antioxidant activity using the DPPH method demonstrated notable radical scavenging capability (IC_{50} = 46.1 ± 1.5 µg/mL), with the activity strongly associated with phenolic and flavonoid levels. Overall,

the findings support the use of orange peel by-products as an inexpensive, sustainable, and effective source for producing natural antioxidants.
The study contributes to advancing circular bio-economy frameworks by transforming agro-industrial waste into high-value products, reducing dependence on synthetic additives, and mitigating the environmental burdens associated with citrus waste disposal.

4.2 Recommendations

Based on the findings, the following practical steps are recommended to enhance the application and industrial scalability of this research:
1. Optimization of Extraction Parameters: Apply advanced statistical models such as Response Surface Methodology (RSM) to refine solvent concentration, solid–liquid ratio, and temperature for maximum recovery efficiency and minimal solvent use.
2. Scale-Up and Solvent Recovery: Conduct pilot-scale extraction trials incorporating solvent recovery systems (e.g., rotary distillation or membrane filtration) to minimize cost and waste generation.
3. Isolation and Bioactivity Testing: Fractionate the ethanol extract using column chromatography or preparative HPLC to isolate active compounds (e.g., hesperidin, naringenin) for detailed pharmacological and cytotoxicity studies.
4. Toxicity and Safety Evaluation: Perform in vitro and in vivo toxicity tests according to OECD guidelines to ensure extract safety for potential food, cosmetic, or nutraceutical applications.
5. Functional Product Integration: Evaluate the stability and performance of orange peel extract when incorporated into food preservatives, cosmetic formulations, or nutraceuticals, focusing on shelf-life and sensory properties.
These recommendations aim to bridge laboratory-scale findings with industrial implementation, promoting both environmental sustainability and economic feasibility.

4.3 Future Perspectives

The green extraction strategy developed in this work can serve as a model for valorizing other agro-industrial residues such as pomegranate, banana, and grape peels. To ensure real-world applicability, Life Cycle Assessment (LCA) and Techno-Economic Analysis (TEA) should be integrated into future research to evaluate environmental impact and economic viability.
Recent LCA studies (e.g., Umenweke et al., 2023; Šafranko et al., 2021) have shown that citrus waste valorization using green solvents significantly reduces greenhouse gas emissions compared to conventional extraction. Similarly, TEA analyses (Singh et al, 2022) demonstrated that solvent recovery and energy-efficient drying can lower production costs by up to 40 %, confirming the scalability of such processes.
The ultimate vision is to establish zero-waste biorefineries where citrus by-products are fully converted into natural antioxidants, essential oils, and dietary supplements, reinforcing the link between environmental protection and sustainable industrial growth.

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