Original Research Article

J. Funct. Mater. Biomol. 9 (1) (2025) pp 893-901

SACRED HEART RESEARCH PUBLICATIONS

Journal of Functional Materials and Biomolecules

Journal homepage: www.shcpub.edu.in

ISSN: 2456-9429

COMPARATIVE EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF CITRUS FRUIT SEED MIXTURES

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Received on 10 March 2025, accepted on 17 April 2025,
Published online on June 2025

Abstract

Roots, herbs, seeds of oil, and cereal crops, fruits, vegetable leaves, and spices have been reported as sources of natural antioxidant compounds. Positive results on the germination of seeds of citrus plants have also been reported, as well as sources of antioxidants in grape seeds Citrus fruits and their juices are evident bearers of certain secondary metabolites particularly antioxidative agents such as vitamin C, flavanones, some phenolics, and fruit pectin which are relevant in nutrition. The bioactive potential of citrus fruit seeds, particularly their antibacterial and antioxidant properties, has drawn significant attention in recent years. This review explores the comparative antibacterial and antioxidant activities of various combinations of citrus seed extracts. Key compounds such as flavonoids, limonoids, and phenolics are highlighted for their roles in bioactivity. The review examines factors influencing efficacy, including extraction methods, seed combinations, and solvent systems. Insights into the practical applications of these mixtures in food preservation, pharmaceuticals, and cosmetics are also provided. Future directions are proposed to optimize these benefits and expand their industrial applications.

Keywords: Citrus seeds, antibacterial activity, antioxidant activity, flavonoids, bioactive compounds, food preservation, extraction methods.

1. Introduction

Fruits of citrus trees come from the family of Rutaceae. It comprises of about 140 genera and 1,300 species, and are quite possibly the most widely grown throughout the world. Citrus fruits are pleasing to eat not only due to their nutritional content but also their taste. The consumption of fruit seeds on the other hand comes with some health benefits and their ability to ward off some diseases. Citrus fruits, including lemons, oranges, grapefruits, and limes, are widely recognized for their rich content of bioactive compounds. While the juice and peel are commonly utilized, the seeds are often discarded despite being reservoirs of valuable secondary metabolites such as flavonoids, alkaloids, and polyphenols. Recent studies highlight the potential of citrus seed (Figure-1) extracts for antibacterial and antioxidant applications. Understanding the synergistic effects of mixtures of these seeds provides opportunities for enhanced bioactivity, making them valuable for industries like food preservation and pharmaceuticals (1-3).

1.1 Historical Usage of Citrus: Citrus fruits have a long history of use in traditional medicine and as natural remedies for various ailments, attributed primarily to their rich phytochemical content. Seeds, though often discarded as by-products, harbour concentrated bioactive compounds.

1.2 Bioactive Compounds in Seeds: Citrus seeds contain an array of bioactive constituents, including polyphenols, flavonoids, limonoids, alkaloids, and essential oils. These compounds contribute to their potential health benefits, especially in oxidative stress mitigation and microbial

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inhibition.

1.3 Rising Interest in Natural Preservatives: The food and pharmaceutical industries increasingly seek natural alternatives to synthetic additives. Citrus seed extracts are emerging as promising candidates due to their dual antibacterial and antioxidant properties.

1.4 Synergistic Potential of Seed Mixtures: Combining different citrus seed extracts may enhance their bioactivity due to synergistic interactions among the diverse phytochemical profiles of each seed type. This concept has inspired research into optimized seed mixtures for specific applications.

1.5 Environmental Sustainability: Utilizing citrus seeds, typically discarded as waste, aligns with the principles of circular economy and sustainable resource management. This approach not only reduces waste but also adds value to the citrus processing industry.

1.6 Global Citrus Production: As one of the most widely cultivated fruit groups worldwide, citrus production generates significant quantities of seeds annually, presenting an underutilized yet abundant resource for bioactive extraction.

1.7 Importance of Extraction Techniques: The efficacy of bioactive compound recovery depends on the extraction method employed. Techniques such as Soxhlet extraction, ultrasonic-assisted extraction, and cold pressing significantly influence the yield and quality of the extracted compounds.

1.8 Broad-Spectrum Applications: Citrus seed mixtures are versatile, finding applications across multiple industries. In addition to food preservation, they are used in cosmetics for anti-aging formulations, in medicine as natural antibiotics, and in packaging as antioxidants.

1.9 Consumer Health and Awareness: With increasing consumer demand for natural and clean-label products, citrus seed extracts offer a plant-based solution, aligning with trends in health-conscious consumerism.

Figure-1

1.10 Research Gaps and Opportunities: While individual seed extracts have been extensively studied, the combined effects of seed mixtures remain underexplored. This creates a fertile ground for innovative research to maximize their bioactive potential.

This review investigates the comparative antibacterial and antioxidant properties of mixtures of citrus seeds, addressing the importance of extraction methods, bioactive profiles, and potential applications (4-8).

2. Phytochemical Composition of Citrus Seeds

Citrus seeds are rich in bioactive compounds, which are pivotal in determining their antibacterial and antioxidant activities:

- **Flavonoids**: Key contributors to antioxidant activity by scavenging free radicals and reducing oxidative stress.
- **Phenolic Acids**: Known for their antimicrobial properties against a range of bacterial pathogens.
- **Limonoids**: Exhibit potent bioactivity, including anti-inflammatory and antimicrobial effects.
- **Essential Oils**: Contain terpenes and other aromatic compounds that enhance antibacterial efficacy.

Table 1: Major Phytochemicals Found in Citrus Seeds

Phytochemical Class	Examples	Biological Activity	Industrial Applications
Flavonoids	Hesperidin, Naringin	Antioxidant, Anti-inflammatory	Nutraceuticals, Functional Foods
Limonoids	Limonin, Nomilin	Anticancer, Antimicrobial	Pharmaceuticals
Alkaloids	Synephrine, Octopamine	Analgesic, Anti-inflammatory	Pain Management, Drug Formulations
Tannins	Catechins, Gallotannins	Astringent, Wound Healing	Cosmetics, Medical Dressings
Essential Oils	Limonene, Linalool	Antimicrobial, Deodorizing	Cleaning Products, Aromatherapy

1. Overview of Phytochemicals in Citrus Seeds

Citrus seeds are an abundant source of bioactive phytochemicals, including flavonoids, limonoids, alkaloids, essential oils, tannins, and carotenoids. These compounds exhibit a wide range of biological activities, such as antioxidant, antimicrobial, and anti-inflammatory properties. Flavonoids, such as hesperidin and naringin, are particularly abundant and are well-known for their free radical-scavenging abilities. Limonoids, another prominent class of compounds found in citrus seeds, have demonstrated potent anti-cancer and cholesterol-lowering effects, making them highly sought after in nutraceutical research.

2. Antioxidants in Citrus Seeds

The antioxidant potential of citrus seeds is primarily attributed to their rich polyphenolic and flavonoid content. These compounds neutralize reactive oxygen species (ROS), preventing cellular damage and reducing the risk of chronic diseases such as cardiovascular ailments and neurodegenerative disorders. For example, phenolic acids like ferulic acid and caffeic acid found in citrus seeds have shown superior efficacy in mitigating oxidative stress compared to synthetic antioxidants.

3. Limonoids and their Therapeutic Potential

Limonoids are highly bioactive terpenoid compounds present in citrus seeds. They contribute to the plant's defense mechanisms and possess significant health benefits for humans. Studies have shown that limonoids exhibit anticarcinogenic, antiviral, and antimicrobial properties. These compounds also play a role in regulating lipid metabolism, making them potential candidates for managing obesity and related metabolic disorders.

4. Alkaloids and Their Bioactivity

Citrus seeds also contain alkaloids, which are nitrogen-containing compounds known for their pharmacological activities. These include anti-inflammatory and analgesic effects. The alkaloids in citrus seeds, while present in smaller quantities than flavonoids and limonoids, have shown synergistic effects when combined with other phytochemicals, enhancing the overall bioactivity.

5. Essential Oils and Aromatic Compounds

The essential oils extracted from citrus seeds are composed of monoterpenes and sesquiterpenes, including limonene, linalool, and citronellol. These compounds are highly valued in the cosmetic and aromatherapy industries for their pleasant aroma and skin-beneficial properties. Additionally, essential oils from citrus seeds exhibit antimicrobial activity, making them effective in natural preservative formulations.

6. Tannins and Carotenoids

Tannins in citrus seeds contribute to their astringent properties and play a crucial role in wound healing and

antimicrobial defense. Carotenoids, on the other hand, are known for their role in protecting against UV-induced oxidative damage and promoting eye health. The presence of these compounds highlights the multifunctional nature of citrus seeds.

6. Variability Across Citrus Species

The phytochemical composition of citrus seeds can vary significantly across species and cultivation conditions. Factors such as soil quality, climate, and harvesting practices influence the concentration and diversity of phytochemicals. For example, the seeds of bitter orange (Citrus aurantium) are particularly rich in alkaloids, whereas sweet orange (Citrus sinensis) seeds have a higher concentration of limonoids and flavonoids.

7. Applications in Food and Medicine

Due to their diverse phytochemical profile, citrus seeds are used in functional foods, dietary supplements, and pharmaceuticals. Their ability to enhance the shelf life of food products as natural preservatives and their use in developing anti-inflammatory and antimicrobial therapies underscore their industrial relevance.

8. Sustainability and Utilization

As citrus seeds are typically discarded during fruit processing, their utilization for phytochemical extraction aligns with sustainable practices. This approach minimizes waste and provides an eco-friendly alternative for sourcing valuable bioactive compounds (9-13).

Antibacterial Activity of Citrus Seed Mixtures

Table 2: Antibacterial Activity of Citrus Seed Extracts

Citrus Species	Extract Tested	Bacterial Strains Targeted	Inhibition Zone (mm)	Minimum Inhibitory Concentration (MIC)
Citrus sinensis	Ethanolic extract	*E. coli*, *S. aureus*	15.2 ± 0.4, 12.8 ± 0.5	125 µg/mL, 250 µg/mL
Citrus aurantium	Methanolic extract	*P. aeruginosa*, *L. monocytogenes*	18.3 ± 0.6, 14.7 ± 0.3	100 µg/mL, 200 µg/mL
Citrus limon	Aqueous extract	*B. subtilis*, *K. pneumoniae*	12.5 ± 0.5, 13.2 ± 0.4	150 µg/mL, 300 µg/mL

Mechanisms of Action

1. Cell Wall Disruption: Bioactive compounds interfere with bacterial cell wall synthesis, leading to structural damage.
2. Protein Inhibition: Certain flavonoids bind to bacterial enzymes, inhibiting essential metabolic pathways.
3. ROS Generation: Compounds such as limonoids induce oxidative stress in bacterial cells.

Comparative Analysis

- Studies show that seed mixtures demonstrate synergistic effects, enhancing antibacterial efficacy compared to individual seeds.
- Orange and lime seed extracts are particularly effective against Escherichia coli and Staphylococcus aureus.
- Variations in antibacterial activity depend on the extraction solvent; ethanol extracts often yield higher bioactivity.

Antioxidant Activity of Citrus Seed Mixtures

Table 3: Antioxidant Activity of Citrus Seed Extracts

Assay Type	Citrus Species	Extract Tested	Activity (IC50)	Reference Standard

DPPH Free Radical Scavenging	Citrus sinensis	Ethanolic extract	45 µg/mL	Ascorbic Acid
ABTS Radical Scavenging	Citrus aurantium	Methanolic extract	30 µg/mL	Trolox
Ferric Reducing Antioxidant Power (FRAP)	Citrus limon	Aqueous extract	60 µmol Fe2+/g dry weight	Quercetin

Mechanisms of Action

1. **Radical Scavenging**: Citrus seeds neutralize free radicals, reducing cellular oxidative damage.
2. **Metal Chelation**: Phenolic compounds chelate transition metals, preventing oxidative chain reactions.

Comparative Analysis

- Lemon and grapefruit seed mixtures show superior antioxidant activities, attributed to higher flavonoid content.
- The method of extraction significantly influences antioxidant potential, with ultrasonic-assisted extraction providing optimal yields.

Factors Influencing Bioactivity

1. **Extraction Method**: Techniques such as Soxhlet extraction and cold pressing yield different concentrations of bioactives.
2. **Seed Combination Ratios**: The proportional mixing of seeds affects the overall bioactivity due to the synergistic interaction of compounds.
3. **Solvent Type**: Polar solvents like ethanol enhance the extraction of phenolic compounds, while non-polar solvents are more effective for oils.

3. Applications

Table 4: Industrial Applications of Citrus Seed Phytochemicals

Industry	Application	Phytochemical Utilized	Purpose
Food	Natural Preservatives	Flavonoids, Tannins	Extend Shelf Life, Reduce Spoilage
Pharmaceuticals	Antimicrobial Drugs	Limonoids, Alkaloids	Treat Infections
Cosmetics	Anti-Aging Products	Flavonoids, Essential Oils	Reduce Oxidative Damage
Agriculture	Biopesticides	Essential Oils, Alkaloids	Natural Pest Control
Energy	Biodiesel Production	Citrus Seed Oil	Sustainable Energy Source

1. Food Preservation and Shelf-Life Enhancement: Citrus seed extracts, rich in antioxidants and antimicrobial compounds, are extensively used as natural preservatives in the food industry. These extracts can inhibit microbial growth and oxidative spoilage, which are major causes of food deterioration. The incorporation of citrus seed-based additives into packaging materials provides an eco-friendly approach to maintaining freshness and extending the shelf life of perishable goods.

2. Nutraceuticals and Functional Foods: The bioactive compounds in citrus seeds, such as flavonoids and limonoids, are formulated into dietary supplements and functional foods. These nutraceuticals are marketed for their health benefits, including improved cardiovascular health, immune support, and anti-inflammatory

properties. Regular consumption of these products can help mitigate the risks of chronic diseases like diabetes and hypertension.

3. Cosmetic Industry Applications: Citrus seed oils and extracts are valued in the cosmetic industry for their skin-nourishing and anti-aging properties. These ingredients are incorporated into creams, lotions, and serums for their ability to combat free radicals, enhance collagen production, and promote skin elasticity. Additionally, their antimicrobial properties make them suitable for acne treatment formulations.

4. Pharmaceutical Applications: Citrus seed phytochemicals have shown promising results in the development of antimicrobial and anticancer agents. Limonoids, in particular, exhibit cytotoxic properties that make them potential candidates for chemo preventive drugs. The anti-inflammatory and analgesic effects of alkaloids derived from citrus seeds are also being explored in pain management therapies.

5. Eco-Friendly Pest Control: The essential oils and tannins from citrus seeds are effective natural pesticides and insect repellents. They are used in agriculture as an alternative to synthetic chemicals, which pose environmental and health hazards. These natural compounds are particularly useful in organic farming, where sustainability and ecological balance are prioritized.

6. Applications in Animal Feed: Citrus seed extracts are incorporated into animal feed as a source of natural antioxidants. These additives improve the nutritional quality of feed and enhance the health of livestock by boosting their immune systems and reducing oxidative stress. This application is gaining traction in the poultry and aquaculture industries.

7. Development of Biodegradable Materials: The use of citrus seed extracts in the production of biodegradable films and coatings is an innovative application in the packaging industry. These materials not only extend the shelf life of products but also address environmental concerns by reducing plastic waste.

8. Natural Cleaning Products: Citrus seed oils are increasingly used in the formulation of eco-friendly cleaning agents. Their antimicrobial and deodorizing properties make them effective in household cleaners, disinfectants, and laundry detergents. These products appeal to consumers seeking sustainable and non-toxic alternatives.

9. Antioxidant Additives in Beverages: Citrus seed extracts are employed in the beverage industry to enhance the antioxidant content of drinks. Functional beverages fortified with these extracts are marketed for their health benefits, catering to the growing demand for wellness-oriented products.

10. Biotechnology and Research: The phytochemicals in citrus seeds serve as model compounds in biotechnological research. They are studied for their potential in drug development, biosensor design, and nanotechnology applications. Researchers are also exploring their role in gene expression modulation and metabolic engineering.

11. Applications in Sustainable Energy: The oil content in citrus seeds can be converted into biodiesel, providing a renewable energy source. This application supports the diversification of energy portfolios and contributes to reducing the dependency on fossil fuels.

12. Anti-Corrosion Agents: Citrus seed extracts are used in industrial applications as eco-friendly corrosion inhibitors. These inhibitors protect metal surfaces in pipelines, machinery, and storage tanks, offering a sustainable alternative to chemical-based solutions.

13. Veterinary Medicine: The antimicrobial and anti-inflammatory properties of citrus seed compounds are utilized in veterinary medicine for treating infections and wounds in animals. These natural remedies are particularly appealing in organic and sustainable farming practices.

14. Research in Weight Management Solutions: Citrus seed compounds like limonoids have been linked to improved lipid metabolism. As a result, they are being

explored for their role in weight management products and metabolic health supplements.

15. Contribution to Circular Economy: The utilization of citrus seeds, a by-product of the juice and food processing industries, embodies the principles of the circular economy. By transforming waste into valuable products, this approach enhances sustainability and adds economic value to agricultural production (14-38).

4. Challenges and Limitations

1. **Standardization**: Variability in seed composition and extraction methods poses challenges in replicating results.
2. **Scalability**: Industrial-scale production requires optimization to maintain bioactivity.
3. **Toxicity Concerns**: High doses of some extracts may exhibit cytotoxic effects, necessitating thorough safety evaluations (39-40).

5. Conclusion

Citrus seed mixtures hold immense potential as antibacterial and antioxidant agents, offering sustainable and natural alternatives for various industries. While current research demonstrates promising results, future efforts should focus on standardizing extraction protocols, understanding synergistic effects, and scaling up production for commercial applications. The integration of citrus seed extracts into food, pharmaceutical, and cosmetic products aligns with global trends toward sustainability and natural product use.

The findings on the antibacterial and antioxidant activities of citrus fruit seed mixtures underscore their significant potential in addressing global health and industrial challenges. Citrus seeds, often considered waste, represent a sustainable resource rich in bioactive compounds, offering a dual solution to food preservation and health enhancement. The synergistic effects observed in seed mixtures further amplify their efficacy, demonstrating the value of integrative approaches in natural product research. Despite their promise, challenges such as optimizing extraction methods, ensuring consistent bioactivity, and addressing potential safety concerns must be overcome to fully realize their potential. Future research should focus on refining these processes, exploring diverse applications, and understanding the mechanisms underlying their bioactivity. By leveraging these insights, citrus seed mixtures can transition from underutilized by-products to valuable components in pharmaceuticals, food, and cosmetic industries, reflecting a harmonious blend of innovation, sustainability, and health advancement.

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