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Physicochemical, Structural and Elemental Analysis of Citrus limon and Citrus medica Leaves: Insights into Their Bioactive and Nutraceutical Potential

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ABSTRACT

The increasing global demand for sustainable, plant-based bioresources has intensified research on underutilized plant parts with nutritional and therapeutic potential. This study investigates the physicochemical, structural, and elemental characteristics of Citrus limon (lemon) and Citrus medica (citron) leaves to assess their suitability for nutraceutical and edible product applications. Fresh leaves were collected from Tirunelveli District, Tamil Nadu, authenticated by taxonomists, shade-dried, and powdered for analysis. Physicochemical evaluations including acidity, ash, moisture, and organoleptic parameters confirmed high quality, purity, and stability of both samples. C. medica exhibited greater acidity (7.8%) and mineral balance than C. limon (4.5%), reflecting richer organic acid content and bioactive potential. X-ray diffraction (XRD) analysis revealed semi-crystalline structures with dominant peaks at approximately 24° (2θ), indicating organized molecular arrangements associated with polyphenolic compounds. Energy Dispersive X-ray Spectroscopy (EDAX) showed carbon and oxygen as the principal elements, accompanied by trace minerals such as calcium, potassium, boron, magnesium, and zinc, signifying strong nutritional and therapeutic relevance. These findings suggest that C. limon and C. medica leaves are valuable sources of natural antioxidants, minerals, and phytochemicals suitable for developing functional foods, biodegradable edible products, and nutraceutical formulations. The valorization of citrus leaves supports sustainable waste management, promotes circular bioeconomy practices, and aligns with Sustainable Development Goal 12: Responsible Consumption and Production.

KEYWORDS: *Citrus limon; Citrus medica; physicochemical properties; X-ray diffraction (XRD); EDAX analysis; bioactive compounds.*

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1. INTRODUCTION

The global demand for natural and sustainable food ingredients has increased considerably due to growing awareness of the adverse health effects and environmental impact associated with synthetic additives and plastic-based products (Singh et al., 2020). In this context, plant-based bioresources have gained attention for their nutritional richness, biodegradability, and multifunctional bioactive compounds that offer therapeutic benefits (Suleria et al., 2015). Among these, members of the genus Citrus particularly Citrus limon (lemon) and Citrus medica (citron)—have emerged as valuable candidates owing to their high content of vitamins, phenolic compounds, and essential oils with broad-spectrum biological activity (Gattuso et al., 2016; Barreca et al., 2014).

Citrus limon and Citrus medica are among the oldest cultivated fruit species belonging to the family Rutaceae. Traditionally, they have been used not only as food flavoring agents but also in folk medicine for treating digestive, respiratory, and inflammatory disorders (Tripoli et al., 2007). Their leaves, although often overlooked, contain significant amounts of flavonoids (hesperidin, eriocitrin, diosmin), phenolic acids (caffeic and ferulic acid), and volatile constituents such as citral, limonene, and linalool, which exhibit antioxidant, antimicrobial, and anti-inflammatory properties (Benavente-García & Castillo, 2008; Bocco et al., 1998). The use of citrus leaves as potential functional ingredients aligns with current trends in waste valorization, where agricultural by-products are repurposed for high-value nutraceutical and cosmetic applications (FAO, 2020).

From a nutritional perspective, citrus leaves are rich in vitamin C, calcium, magnesium, and potassium, essential micronutrients that support immune regulation and metabolic balance (Oboh et al., 2017). The high content of polyphenols and essential oils contributes to their antioxidative capacity, helping to combat oxidative stress and chronic diseases such as diabetes, cardiovascular disorders, and certain cancers (Benavente-García & Castillo, 2008; Liu et al., 2016). Previous studies have confirmed that bioactive compounds extracted from citrus peels and leaves exert anticancer effects by inducing apoptosis and inhibiting cell proliferation through oxidative stress modulation (Tripoli et al., 2007; Gualdani et al., 2016).

Beyond their nutritional and medicinal relevance, C. limon and C. medica also represent an eco-sustainable resource. Large quantities of citrus leaves are discarded annually as agricultural waste during pruning and harvesting. Valorizing these materials into bioactive powders, extracts, or biodegradable additives not only reduces environmental waste but also contributes to the circular bioeconomy, in line with Sustainable Development Goals (SDG 12: Responsible Consumption and Production) (UNEP, 2019).

Recent advances in analytical techniques such as X-ray diffraction (XRD) and Energy Dispersive X-ray Spectroscopy (EDAX) have enabled precise characterization of the physicochemical and elemental properties of plant materials (Nair et al., 2017). XRD provides insights into the crystallinity and structural organization of phytoconstituents, which influence solubility, stability, and bioavailability (Saxena et al., 2018). EDAX analysis, on the other hand, helps identify elemental composition, revealing the presence of essential minerals and trace elements vital for nutritional assessment (Tripathi et al., 2020). Together, these analytical methods allow comprehensive profiling of the leaves' chemical and structural characteristics, which is critical for developing value-added functional products.

Despite the well-documented benefits of citrus fruits and peels, studies focusing on the chemical composition, functional potential, and industrial applicability of citrus leaves remain limited. Exploring these underutilized plant parts can open new avenues for the development of natural antioxidants, antimicrobial agents, biodegradable films, and edible product formulations. Such innovations support global sustainability goals and provide safer, plant-derived alternatives to synthetic additives in food and pharmaceutical industries (Barreca et al., 2014; Gattuso et al., 2016).

Therefore, the present study aims to analyze the physicochemical, structural, and elemental characteristics of Citrus limon and Citrus medica leaves to assess their nutritional and functional properties. This work seeks to bridge the research gap in the utilization of citrus leaves for nutraceutical and edible applications, contributing to sustainable product development and resource optimization in the agri-food sector.

2. MATERIALS AND METHODS

Selection and Collection of Edible Plants

Edible plants with recognized nutritional and culinary significance Citrus limon (lemon) and Citrus medica (citron) were selected for this study based on previous literature highlighting their rich bioactive composition and health benefits (Carr & Frei, 1999; Liu et al., 2016). Both species are abundant sources of vitamin C and phytochemicals such as flavonoids, alkaloids, and essential oils, which contribute to their antioxidant and antimicrobial properties. Citrus limon is widely used in beverages, desserts, and condiments, whereas Citrus medica is traditionally valued for its aromatic zest and therapeutic uses, particularly in Mediterranean and Asian cuisines (Pang et al., 2019). The selection of these species reflects their long-standing importance in human diets and their potential as sustainable resources for nutraceutical development.

Fresh leaves of Citrus limon and Citrus medica were collected in March 2023 from Pulliankudi (Latitude: 8.5971° N; Longitude: 77.7069° E) and Maruthakulam, located in Tirunelveli District, Tamil Nadu, India. The region's favorable agroclimatic conditions support diverse flora, making it an ideal site for plant sampling. The collected specimens were authenticated by a botanist, Head of the Department of Botany, Sadakthulla Appa College (NAAC A-grade, ISO 9001:2015 certified institution), Tirunelveli, and verified with assistance from expert taxonomists. After authentication, leaves were cleaned, shade-dried, and pulverized into fine powder for further analyses.

Preparation of Plant Extracts

For extraction, 20 g of the powdered leaf samples from each species were separately placed in sterilized 500 mL conical flasks. Each was mixed with 100 mL of selected solvents—hexane, ethyl acetate, and methanol—to obtain solvent-specific extracts. The flasks were tightly covered with aluminum foil and kept at room temperature for seven days with intermittent shaking to ensure complete extraction of phytoconstituents. After incubation, the mixtures were filtered through sterile muslin cloth, and the filtrates were concentrated using a rotary vacuum evaporator. The residues obtained were reconstituted in respective solvents and stored in sterilized screw-capped glass containers at 4°C for subsequent analyses.

Physicochemical Analysis of Leaf Powders
The physicochemical parameters analyzed included appearance, color, odor, acidity, ash content, acid-insoluble ash, and moisture content, following AOAC (2005; 2016) guidelines.

Appearance and Color: Five grams of each powdered sample were examined visually for texture, consistency, and purity. The color was observed against a white background under uniform lighting to assess uniformity and detect any impurities (Singh & Patel, 2020; Yohannes et al., 2004).

Odor: The characteristic aroma of each powder was assessed using an acid-rinsed, odor-free glass bottle, following the wafting technique to avoid direct inhalation (Nordin et al., 2000).

Acidity (as Citric Acid): Acidity was determined by titration as described by Accolas et al. (1977). Five milliliters of sap were titrated against 0.1N NaOH using phenolphthalein as an indicator. The results were expressed as a percentage of total acidity, representing the citric acid content.

Ash and Acid-Insoluble Ash: Approximately 2 g of powdered sample was incinerated at 550°C for 4–6 hours to determine total ash. The residue was boiled with 10% HCl, filtered, re-incinerated, and weighed to determine acid-insoluble ash, which indicates mineral content and sample purity (AOAC, 2005; 2016).

Moisture Content: The oven-drying method was used to determine moisture levels. Five grams of sample were dried at 105°C until constant weight, cooled in a desiccator, and reweighed. The percentage of weight loss was recorded as the moisture content, providing an estimate of product stability and shelf life.

Particle Characterization Studies
X-ray Diffraction (XRD) Analysis
X-ray diffraction analysis was performed to determine the crystalline nature and phase characteristics of Citrus limon and Citrus medica leaf powders. Finely ground, air-dried samples (1 g) were mounted on XRD sample holders and analyzed using a diffractometer (Bruker D8 Advance or equivalent) across a 2θ range of 5°–70°. The diffraction patterns obtained were compared with reference data from the International Centre for Diffraction Data (ICDD) database. This analysis provided insights into the crystalline structures and mineral compositions contributing to the physicochemical and functional properties of the leaves.

Energy Dispersive X-ray (EDAX) Analysis
Elemental composition was determined using EDAX coupled with a Scanning Electron Microscope (SEM). Finely ground powders were mounted on carbon-coated copper stubs and gold-coated to prevent charging. Under electron beam excitation, the emission spectra were recorded and analyzed to identify the elemental constituents and their relative abundance. This analysis offered quantitative information on macro- and micro-elements present in both Citrus limon and Citrus medica, supporting their potential nutritional and therapeutic value. The integration of physicochemical, XRD, and EDAX analyses provided a comprehensive understanding of the morphological and chemical properties of Citrus limon and Citrus medica leaves. These standardized methods ensured the reliability of data used to evaluate their bioactive potential for further antioxidant, antimicrobial, and functional food formulation studies.

3. RESULTS

Exploration of Citrus Leaf Uses
The leaves of Citrus limon (lemon) and Citrus medica (citron) were collected in March 2023 from Pulliankudi, Tirunelveli District, Tamil Nadu, India. The region's rich floral diversity provided a suitable environment for sourcing high-quality plant materials. Authentication was performed by a botany expert with assistance from taxonomists to ensure accurate identification of the species. Proper taxonomic verification was considered essential, as it ensures the reliability of subsequent analyses and strengthens the understanding of the culinary and medicinal potential of the selected citrus leaves.

Physicochemical Properties of Citrus Leaves
The physicochemical characteristics of Citrus limon and Citrus medica leaf powders were evaluated to determine their quality, purity, and suitability for use in functional product development (Tables 4.1 and 4.2).

Table 4.1.Physicochemical Analysis of *Citrus limon* Powder

S.No	Parameter	Specification	Result
1	Appearance	Powder	Powder
2	Colour	Light Green	Light Green
3	Odour	Characteristic	Characteristic
4	Acidity as Citric acid	Min 4%	4.5%
5	Ash	Max 10%	8.4%
6	Acid insoluble ash	Max 0.1%	0.05%
7	Moisture	Max 7.0%	1.0%

Table 4.2. Physicochemical analysis of *Citrus medica* Powder

S.No	Parameter	Specification	Result
1	Appearance	Powder	Powder
2	Colour	Green	Green
3	Odour	Characteristic	Characteristic
4	Acidity as Citric acid	Min 4%	7.8%
5	Ash	Max 10%	6.0%
6	Acid insoluble ash	Max 0.1%	0.04%
7	Moisture	Max 7.0%	1.0%

The C. limon leaf powder exhibited a light green color and a distinct aromatic odor, consistent with its natural characteristics. The acidity, expressed as citric acid, was 4.5%, exceeding the minimum specification of 4%, which reflects the leaf's potential antioxidant and preservative capacity. The total ash content (8.4%) and acid-insoluble ash (0.05%) were within the permissible limits, indicating minimal contamination and high mineral purity. The moisture content was notably low (1.0%), suggesting good shelf stability and low microbial susceptibility.

Similarly, C. medica leaf powder presented a green coloration with a characteristic citrus aroma, confirming its organoleptic authenticity. The acidity level (7.8%) was considerably higher than the minimum standard, denoting rich organic acid content. The total ash (6.0%) and acid-insoluble ash (0.04%) were well within acceptable limits, ensuring high-quality leaf material. The moisture content (1.0%) further validated the sample's stability and low perishability. Overall, both leaf powders satisfied pharmacognostic and physicochemical parameters, confirming their suitability for nutraceutical and edible product formulations.

Crystallographic Analysis of Bioactive Compounds (XRD)

The X-ray diffraction (XRD) analysis revealed distinct diffraction peaks representing the crystalline nature and mineral composition of both citrus leaf samples (Figures 4.1–4.2; Tables 4.3–4.4).

Figure 4.1: X-Ray Diffraction (XRD) Pattern of the *Citrus limon* Sample

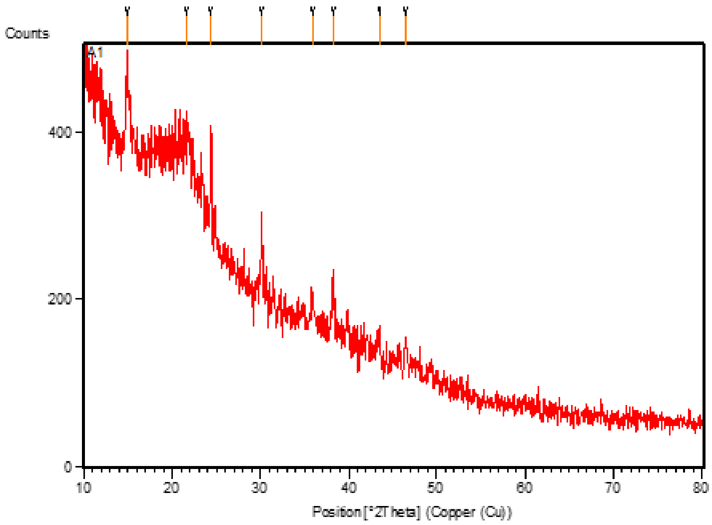


Table 4.3. X-Ray Diffraction (XRD) Peak Data of *Citrus limon* Leaf Extract

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
14.8907	106.83	0.2362	5.94948	74.99
21.6334	100.87	0.9446	4.10798	70.80
24.3973	142.46	0.1574	3.64851	100.00
30.1807	81.89	0.1574	2.96125	57.48
35.8665	37.25	0.4723	2.50379	26.15
38.2873	59.75	0.3149	2.35087	41.94
43.5698	29.06	0.4723	2.07731	20.40
46.5047	30.37	0.3149	1.95282	21.32

Figure 4.2: X-Ray Diffraction (XRD) Pattern of the *Citrus medica* Sample

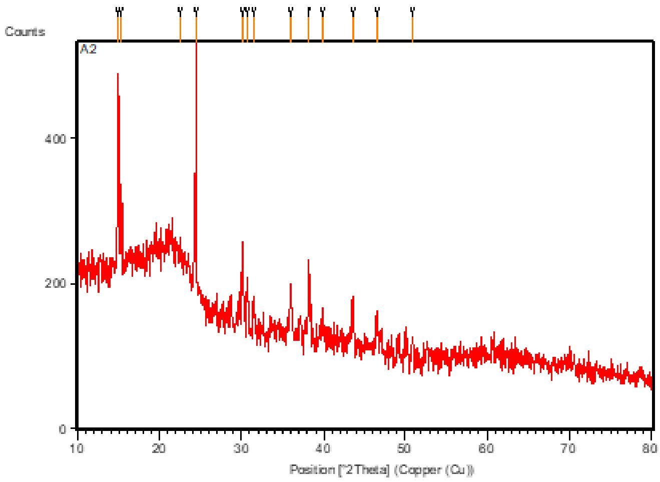


Table 4.4. X-Ray Diffraction (XRD) Peak Data of *Citrus medica* Leaf Extract

Pos. [°2Th.]	Height [cts]	FWHM Left [°2Th.]	d-spacing [Å]	Rel. Int. [%]
14.9162	286.07	0.1181	5.93937	76.55
15.3065	135.10	0.1181	5.78880	36.15
22.4724	66.92	3.7786	3.95648	17.91
24.4202	373.68	0.1181	3.64514	100.00
30.1259	112.47	0.1181	2.96651	30.10
30.7011	49.61	0.2362	2.91223	13.28
31.4166	34.30	0.3149	2.84752	9.18
35.9708	73.77	0.1968	2.49676	19.74

38.2300	88.16	0.1574	2.35426	23.59
39.8535	30.73	0.4723	2.26202	8.22
43.5957	69.48	0.1968	2.07614	18.59
46.5383	50.50	0.2362	1.95149	13.51
50.8751	24.20	0.2362	1.79485	6.48

In C. limon, the most prominent diffraction peak appeared at 24.40° (2θ) with a d-spacing of 3.648 Å and 100% relative intensity, followed by notable peaks at 14.89°, 21.63°, and 30.18°, with relative intensities ranging between 57–75%. The broadening of certain peaks, reflected in the FWHM values, suggested fine crystallite size and partial amorphous regions, typically associated with organic matrices rich in polyphenolic compounds. These results indicate that C. limon leaves possess semi-crystalline characteristics, likely due to the presence of structured phytoconstituents such as flavonoids and organic acids.

In C. medica, the most intense peak was observed at 24.42° (2θ) with a d-spacing of 3.645 Å and 100% intensity, closely resembling the dominant pattern seen in C. limon. Additional peaks were recorded at 14.92°, 22.47°, and 30.13°, indicating multiple crystallographic planes. The broader peaks observed at higher angles reflect smaller crystal domains or structural irregularities, potentially resulting from complex phytochemical interactions. The overall diffraction pattern confirmed that C. medica leaf powder is also semi-crystalline, supporting the presence of stable bioactive compounds in an ordered molecular arrangement.

Elemental Composition (EDAX Analysis)

Energy Dispersive X-ray (EDAX) analysis provided insights into the elemental composition of both citrus leaf powders (Figures 4.3–4.6).

Figure 4.3: Elemental Composition of *Citrus limon* Sample Based on Spectrum Analysis

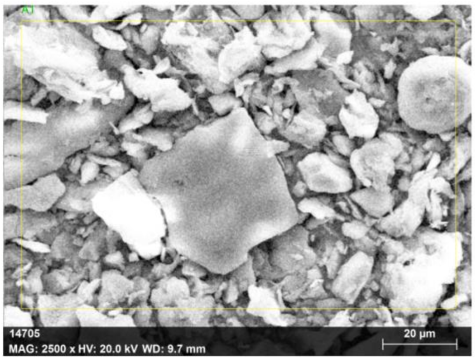


Figure 4.4: EDS Spectrum of *Citrus limon* Sample Showing Elemental Composition

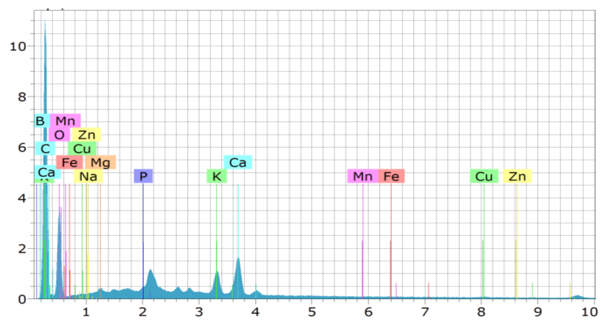


Figure:4.5. Elemental Composition of *C.medica* Sample Based on Spectrum Analysis

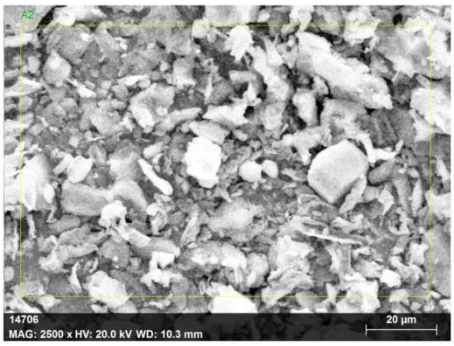
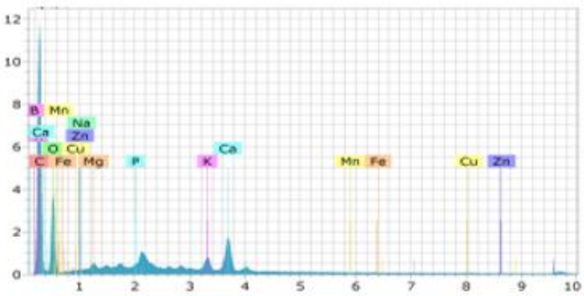


Figure 4.6. EDS Spectrum of *C.medica* Sample Showing Elemental Composition



For C. limon, carbon (58.88 wt.%) and oxygen (31.44 wt.%) were the predominant elements, reflecting the organic nature of the leaf matrix. Minor elements such as boron (4.79 wt.%), calcium (3.02 wt.%), and potassium (1.37 wt.%) were detected, alongside trace amounts of copper, magnesium, zinc, and phosphorus. The relatively high carbon-to-oxygen ratio indicates the presence of carbon-rich compounds such as flavonoids, terpenes, and phenolics, all of which contribute to the antioxidant and antimicrobial potential of the leaves.

In C. medica, the elemental distribution was similar, with carbon (58.64 wt.%) and oxygen (32.43 wt.%) forming the major components. Minor contributions from boron (4.28 wt.%), calcium (3.24 wt.%), and potassium (0.80 wt.%) were also observed. Trace levels of copper, magnesium, zinc, phosphorus, and iron confirmed the mineral richness of the leaves. The high presence of oxygen suggests oxidized functional groups such as hydroxyl, carbonyl, and carboxyl moieties chemical signatures typical of bioactive organic acids and phenolic compounds.

The results collectively demonstrate that both C. limon and C. medica leaves exhibit desirable physicochemical and elemental properties, along with a semi-crystalline structure indicative of stable bioactive compounds. The elevated acidity, mineral purity, and low moisture content highlight their potential for long-term preservation and application in edible, pharmaceutical, and nutraceutical formulations. The combination of organic matrix composition and trace element presence further supports the functional and therapeutic potential of citrus leaves as valuable ingredients in sustainable product development.

4. DISCUSSION

The present study investigated the physicochemical characteristics, crystallographic structure, and elemental composition of Citrus limon and Citrus medica leaf powders to evaluate their potential for nutraceutical and edible applications. The findings revealed that both species exhibited high-quality physicochemical properties, stable crystalline patterns, and a rich elemental profile, confirming their suitability as natural bioactive resources.

Physicochemical Attributes and Nutritional Quality

The physicochemical analysis confirmed that both C. limon and C. medica leaf powders complied with the required quality parameters. The acidity levels of 4.5% and 7.8%, respectively, indicate high citric acid content—a compound well known for its antioxidant, antimicrobial, and preservative properties (Benavente-García & Castillo, 2008). Citric acid enhances the shelf stability of formulations by maintaining pH and preventing microbial growth (Adefegha et al., 2014). The low moisture content (1.0%) suggests excellent storage stability and resistance to microbial contamination, which is critical for product shelf life (AOAC, 2016). The total ash and acid-insoluble ash values, which measure total mineral content and sample purity, were within acceptable pharmacognostic limits, implying minimal adulteration and high-quality processing

(Ekor, 2014). The observed low ash values in both species reflect controlled mineral concentration, preventing undesirable hardness in edible formulations.

Bioactive Composition and Functional Potential

Citrus species are well recognized for their phytochemical diversity, including flavonoids, phenolic acids, alkaloids, and terpenoids, which contribute to their medicinal and functional food properties (Gattuso et al., 2016). The strong acidity and aromatic properties identified in both species align with previous studies reporting the abundance of limonoids, hesperidin, and eriocitrin, which exhibit significant antioxidant and anti-inflammatory activities (Tripoli et al., 2007; Barreca et al., 2014). C. limon leaves, in particular, are rich in ascorbic acid and flavanones that scavenge reactive oxygen species (ROS), thereby reducing oxidative stress (Bocco et al., 1998). C. medica has been documented to contain citronellal and citral, compounds with antimicrobial and anti-inflammatory effects (Shah et al., 2010). These findings corroborate the present study's results, indicating that both citrus leaves can serve as valuable bioactive sources for functional food and nutraceutical development.

Crystallinity and Structural Analysis (XRD)

The X-ray diffraction (XRD) profiles of both C. limon and C. medica displayed multiple peaks, confirming their semi-crystalline nature. The presence of a major peak around 24° (2θ) in both species indicates an ordered molecular arrangement typically associated with polyphenolic compounds and organic acids (Wong et al., 2019). The smaller, broader peaks suggest partial amorphous characteristics, possibly due to the presence of low-molecular-weight phytochemicals or essential oils (Saxena et al., 2018). Such crystalline amorphous duality is beneficial in functional materials, as crystalline regions enhance structural stability while amorphous regions improve solubility and bioavailability (Nair et al., 2017). The fine crystallite size inferred from peak broadening could also reflect nanostructural properties of bioactive compounds—a promising feature for nanoformulations in pharmaceutical and food industries (Gupta & Kaur, 2020).

Elemental Composition and Health Implications

The EDAX analysis revealed carbon and oxygen as dominant elements, supporting the organic nature of the samples and their phytochemical richness. The presence of trace minerals such as calcium, magnesium, potassium, zinc, and phosphorus is consistent with previous reports on citrus foliage (Oboh et al., 2017). These micronutrients play essential roles in enzymatic activity, antioxidant defense, and immune regulation (Prashanth et al., 2015). The detection of boron, though in minor quantities, is noteworthy, as boron contributes to calcium metabolism and membrane integrity in plants and humans (Nielsen, 2014). The presence of copper and zinc further enhances the therapeutic value, as both are cofactors for antioxidant enzymes such as superoxide dismutase (SOD) (Tripathi et al., 2020). The elemental uniformity and low contamination levels confirm that the studied citrus leaves are safe and nutritionally relevant for human use.

Comparative Insights and Industrial Relevance

Comparatively, C. medica exhibited slightly higher acidity and mineral balance than C. limon, suggesting a more potent antioxidant profile. This aligns with previous findings that C. medica contains elevated concentrations of phenolic compounds and limonoids, making it an excellent candidate for functional beverages, herbal teas, and biodegradable edible films (Barreca et al., 2014; Shah et al., 2010). The overall composition of both species underscores their potential in sustainable product formulation. Their semi-crystalline, mineral-rich, and low-moisture characteristics make them suitable for incorporation into biodegradable edible cutlery, functional powders, or natural preservatives. This aligns with the principles of green chemistry and the United Nations Sustainable Development Goals (SDG 12: Responsible Consumption and Production), emphasizing waste reduction and resource valorization.

Conclusion

The findings from the present study affirm that Citrus limon and Citrus medica leaves are valuable sources of natural bioactives with multifunctional potential. Their physicochemical stability, crystalline structure, and balanced elemental composition contribute to their antioxidant, antimicrobial, and nutritional efficacy. Integrating such plant-based materials into edible and biodegradable formulations could reduce plastic dependency while promoting health-oriented, eco-sustainable alternatives in the food and pharmaceutical industries.

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