International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

# EDXRF-Based Trace Elemental Analysis of *Citrus aurantifolia* Leaves with Emphasis on Anticancer Potential

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**Abstract:** ***Cancer remains one of the most leading causes of mortality worldwide, and increasing attention has been directed toward plant-based therapeutics as complementary strategies in cancer management due to its rich bioactive compounds and lower side effects. Trace elements play vital roles in carcinogenesis, antioxidant defense, immune regulation, and cellular metabolism. The present study investigates the trace elemental composition of Citrus aurantifolia leaves using Energy Dispersive X-Ray Fluorescence (EDXRF) spectroscopy, with emphasis on their potential relevance to anticancer activity. EDXRF is a non-destructive analytical technique used for the qualitative and quantitative estimation of elemental composition present in a wide range of materials, including metals, biological samples, soils, and medicinal plants. EDXRF analysis revealed that, the presence of essential macro and microelements including K, Ca, Fe, Zn, Mn, Cu, Sr, and trace levels of Cr, Ni, and Pb. Potassium and calcium were found in higher concentrations, whereas biologically important micronutrients such as Fe, Zn, and Mn were present in moderate amounts. These elements are known to contribute to antioxidant enzyme systems, DNA repair mechanisms, and immune modulation associated with cancer prevention. The results support the therapeutic potential of Citrus aurantifolia leaves and highlight their significance as a natural source of bioactive trace elements for complementary anticancer applications. Future studies should focus on validating the anti-cancer activity via bio-assays, integrate advanced analytical techniques, assess bioavailability and toxicity, and develop standardized Citrus aurantifolia formulations for therapeutic use.***

**Keywords:** *Citrus aurantifolia*, EDXRF analytical technique, Trace elements, Elemental composition, Medicinal plants and Cancer disease

## 1. Introduction

Cancer remains one of the most formidable global health challenges, accounting for a significant proportion of morbidity and mortality worldwide. According to recent global cancer statistics, cancer is responsible for nearly 10 million deaths annually, making it the second leading cause of death after cardiovascular diseases (Nwosu, N., 2024). The burden is rapidly increasing, particularly in low- and middle-income countries such as India, where changing lifestyles, environmental exposures, and population aging contribute significantly to rising incidence rates (Gyasi, R. M., 2020). In India alone, millions of new cancer cases are diagnosed each year, with a high mortality rate largely due to late-stage detection and limited access to advanced treatment facilities. The most prevalent cancers include breast, lung, cervical, and gastrointestinal malignancies, which collectively account for a majority of cancer-related deaths (Kamangar, F., 2006).

Cancer is a multifactorial disease characterized by uncontrolled cell proliferation, evasion of apoptosis, and metastatic spread. Its development involves a complex interplay of genetic mutations, environmental influences, lifestyle habits, and nutritional imbalances (Priya, K., 2026 and Singh, H., 2024). Major risk factors include tobacco consumption, alcohol intake, exposure to carcinogenic chemicals, radiation, chronic infections, and dietary deficiencies (Lewandowska, A. M., 2018). In addition, oxidative stress caused induced by reactive oxygen species (ROS) and chronic inflammation plays a crucial role in the initiation and progression of cancer (Aggarwal, V., 2019). Recent studies also highlight the importance of trace elements in regulating cellular pathways associated with carcinogenesis, including DNA repair, immune response, and antioxidant defense mechanisms.

Conventional cancer treatment strategies primarily include surgery, chemotherapy, radiation therapy, immunotherapy, and targeted therapy. While these approaches have significantly improved survival rates, they are often associated with severe side effects and limitations (Cavalcanti, I. D. L., 2021). Chemotherapeutic drugs, for instance, lack specificity and can damage healthy cells, leading to adverse effects such as immunosuppression, hair loss, gastrointestinal disturbances, and organ toxicity (Carr, C., 2008). Radiation therapy may cause tissue damage to surrounding tissues and increase malignancies. Although targeted therapies and immunotherapies promising outcomes, remain they remain expensive and inaccessible to a large segment of the population (Srinivasan, D., 2024). Furthermore, the development of drug resistance continues to pose a significant challenge in effective cancer management. These limitations highlight the urgent need for alternative or complementary therapeutic strategies that are safer, cost-effective, and associated with minimal side effects.

In this context, medicinal plants have gained substantial attention as promising sources of novel anticancer agents. Traditional systems of medicine, like Ayurveda, have long utilized plant-based remedies for the treatment of various diseases, including cancer (Jha, S. K.,2025). Phytochemicals such as flavonoids, alkaloids, terpenoids, and phenolic compounds are known to exhibit strong anticancer properties through mechanisms such as antioxidant activity, inhibition of cell proliferation, induction of apoptosis, and modulation of cellular signaling pathways (Majrashi, T. A., 2023). In addition to these bioactive organic compounds, essential and trace elements present in medicinal plants plays an important role in enhancing their therapeutic potential.

*Citrus aurantifolia* (commonly known as lime) is a widely distributed medicinal plant belonging to the Rutaceae family. It has been extensively utilized in traditional medicine for its antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Patil, J. R.,2009). Various parts of the plant, including leaves, fruits, and peels, are rich in bioactive compounds such as flavonoids, limonoids, and vitamin C. Recent studies have shown that *Citrus aurantifolia* extracts exhibit cytotoxic effects against a different cancer cell line, including breast, colon, and liver cancers. The anticancer potential of this plant is attributed not only to its phytochemical constituents but also to its mineral composition, which may significantly influence cellular metabolism and immune responses (Patil, J. R.,2010).

Trace elements are indispensable for maintaining normal physiological functions and play a critical role in cancer biology. Elements such as zinc (Zn), selenium (Se), copper (Cu), iron (Fe), manganese (Mn), and calcium (Ca) are involved in enzymatic activities, antioxidant defense mechanisms, and regulation of gene expression (Jomova, K., 2025). For instance, zinc is essential for DNA synthesis and repair, while selenium helps reduce oxidative stress and enhance immune function. However, an imbalance of both deficiency and excess of these elements can have detrimental effects. Deficiency of essential trace elements may impair immune function and increase susceptibility to cancer, whereas excessive accumulation of certain metals, such as copper and iron, can promote oxidative damage and tumor progression (Yildiz, A.,2019). Moreover, toxic elements, even at low concentrations, may contribute to carcinogenesis by inducing DNA damage and disrupting cellular homeostasis.

Therefore, the quantitative analysis of trace elements in medicinal plants is essential for understanding both their therapeutic potential and safety. Energy Dispersive X-Ray Fluorescence (EDXRF) spectroscopy has emerged as a powerful, non-destructive analytical technique for multi-elemental analysis. It allows for the rapid and accurate determination of elemental composition in biological samples without extensive sample preparation (Jyothsna, S.,2020). EDXRF technique is particularly valuable for evaluating the presence of both essential and toxic elements in medicinal plants, thereby providing deeper insights into their pharmacological properties and potential health benefits. In this context, the present study focuses on the EDXRF-based trace elemental analysis of *Citrus aurantifolia* leaves, with special emphasis on their anticancer potential. Biologically By significant elements such as phosphorus (P), sulfur (S), chlorine (Cl), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), selenium (Se), bromine (Br), rubidium (Rb), and strontium (Sr) were identified and quantified to explore their possible correlation with anticancer activity. Furthermore, comparison with standard reference materials was ensures the accuracy and reliability of the obtained results.

The main objective of this research is to evaluate the elemental profile of *Citrus aurantifolia* leaves using EDXRF and to explore the possible role of these elements in cancer prevention and management. This study also seeks to contribute to the growing body of evidence supporting the use of medicinal plants as alternative or complementary therapeutic agents in oncology. By providing a scientific basis for the anticancer potential of *Citrus aurantifolia*, the findings may pave the way for the development of novel, plant-based therapeutic strategies with improved safety and efficacy.

## 2. Materials and Methods

### 2.1 *Sample Collection and Preparation*

Fresh leaves of *Citrus aurantifolia* were collected from healthy plants grown in local agricultural regions as illustrated in the figure 1. The plant species was authenticated by Department of botany, Kakatiya University Warangal. The collected leaves were washed thoroughly with tap water followed by distilled water to remove dust and contaminants. The leaves were shade-dried at room temperature (25–30 °C) for approximately 10–12 days until constant weight was achieved. The dried samples were powdered using a clean agate mortar and pestle to avoid contamination. The powdered material was sieved through a fine mesh (≤75 μm) to obtain uniform particle size. Approximately 150 mg of the powdered sample was pressed into pellets using a hydraulic pellet press under uniform pressure without binder. The prepared pellets were stored in a desiccator prior to analysis.

**Figure 1:** Citrus aurantifolia leaves

### 2.2 Experimental Setup (EDXRF Analysis)

Trace elemental analysis was carried out using an Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer at UGC, DAE-CSR Kolkata. The instrument was equipped with an appropriate X-ray excitation source and a high-resolution solid-state detector for multi-elemental detection (Jyothsna, S.,2020) The prepared pellets were irradiated under optimized experimental conditions to analyze major, minor, and trace elements present in the plant sample. Spectral acquisition time and excitation parameters were selected to achieve high sensitivity and good counting statistics. Elemental concentrations were measured in ppm level (parts per million) and the obtained results were statistically assessed and compared with standard certified reference material NIST 1515 apple leaf to assess elemental enrichment and their possible relevance to the anti-asthmatic therapeutic properties of *Citrus aurantifolia* leaves.

## 3. Results and Discussion

The elemental composition of *Citrus aurantifolia* leaves was quantitatively analyzed using an Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer, and the results are presented in Table 1. The concentrations of biologically significant elements, including P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, and Sr, were determined and compared with the certified reference material NIST 1515 (apple leaf standard) to evaluate the accuracy and reliability of the analytical method. Fig. 2 shows the EDXRF spectrum of *Citrus aurantifolia* leaves.

**Table 1:** The average elemental concentrations in *Citrus aurantifolia* (leaves) compared with standard NIST 1515 apple leaf

| Elements | *Citrus aurantifolia* (leaves) | NIST 1515 apple leaf standard certified values |
|---|---|---|
| P | 820.44±423.46 | 1590 |
| S | 425.06±117.75 | 1800 |
| Cl | 567.32±199.7 | 579 |
| K | 17840.30±681.12 | 16100 |
| Ca | 12460.02±226.96 | 152260 |
| Mn | 205.65±3.92 | 54 |
| Fe | 812.21±7.21 | 83 |
| Cu | 32±1.01 | 5.64 |
| Zn | 92.47±4.54 | 12.50 |
| Se | 0.29±0.24 | 0.05 |
| Br | 7.24±0.45 | 1.80 |
| Rb | 19.85±0.78 | 10.20 |
| Sr | 45.20±0.08 | 25 |

The elemental composition of *Citrus aurantifolia* leaves was quantitatively analyzed using an Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer, and the results were presented in Table 1. The concentrations of biologically significant elements, such as P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, and Sr, were determined and compared with the certified reference material NIST 1515 (apple leaf standard) to evaluate the accuracy and reliability of the analytical technique.

The results indicate that, potassium (K) was recognized as the most abundant macro-elements in *Citrus aurantifolia* leaves (17840.30 ± 681.12 ppm), exceeding the NIST reference value (16100 ppm) indicating its crucial role in plant metabolism and enzymatic functions (Jyothsna, S.,2020). In comparison calcium (Ca) was identified although present inconsiderable amounts (12460.02 ± 226.96 ppm) but remained significantly lower than the standard value (152260 ppm), suggesting due to specific variation and possible environmental influences on mineral uptake (López-Lefebre, L. R., 2001).



Paper ID: SR26421170449 DOI: https://dx.doi.org/10.21275/SR26421170449

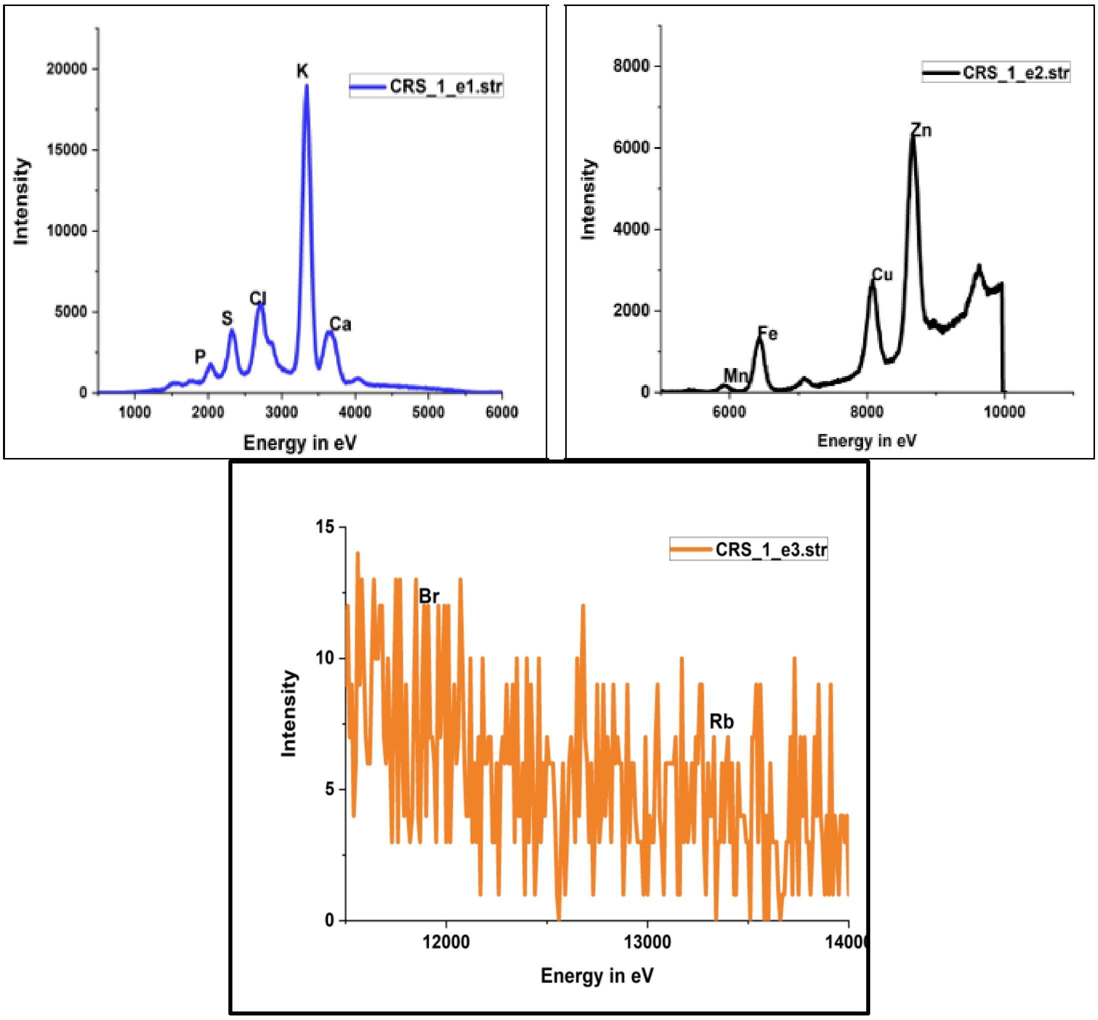


**Figure 2:** EDXRF spectrum of *Citrus aurantifolia* leaves [CRS (*Citrus aurantifolia)* -e1, e2, e3 different energy ranges]

Phosphorus (P) and sulfur (S), which play essential role in energy metabolism and protein synthesis (Lal, M. A.,2023), were estimated relatively at lower concentrations (820.44 ± 423.46 ppm and 425.06 ± 117.75 ppm, respectively) compared to the standard certified values. In contrast, chlorine (Cl) showed a concentration (567.32 ± 199.7 ppm) that nearly matches with certified value of NIST 1515, indicating good analytical reliability and accuracy for this element.

Among the trace elements, manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) were exhibited significantly higher concentrations compared to the NIST 1515 values. In particular, iron (812.21 ± 7.21 ppm vs. 83 ppm) and manganese (205.65 ± 3.92 ppm vs. 54 ppm) showed a remarkable increase, indicating their potential role in enhancing enzymatic functions and redox processes. Similarly, zinc (92.47 ± 4.54 ppm vs. 12.50 ppm) and copper (32 ± 1.01 ppm vs. 5.64 ppm) were also reported at higher amounts, which may contribute to improved antioxidant activity, immune function, and overall physiological benefits (Osredkar, J.,2011). Furthermore, selenium (Se), is also an essential antioxidant trace element, was detected at 0.29 ± 0.24 ppm, which is higher than the standard value (0.05 ppm), indicating its potential role in oxidative stress reduction (Krakowiak, A.,2023). Bromine (Br), rubidium (Rb), and strontium (Sr) were also found in elevated concentrations compared to the standard. These elements, although not primary nutrients, may reflect the geochemical characteristics of the soil and environmental conditions where the plant was grown. Overall, the comparison with NIST 1515 demonstrates good agreement for certain elements, particularly Cl, while notable deviations in other elements may be attributed to differences in plant species, geographical location, soil composition, and matrix effects. The consistency in measurement and detection of multiple elements confirms the reliability and effectiveness of the EDXRF technique for rapid, and non-destructive elemental analysis. The presence of essential macro-and trace elements, particularly K, Fe, Zn, Cu, and Se, underscores the nutritional and medicinal importance of *Citrus aurantifolia* leaves. These elements are known to play key role in enzymatic functions, antioxidant defense, and dermatological health, supporting the traditional use of this plant in therapeutic applications.

## 4. Conclusion

This study demonstrates the effectiveness of EDXRF spectroscopy as a rapid, non-destructive and multi-elemental technique for analyzing the elemental concentration of *Citrus aurantifolia* leaves. The results reveal a rich presence of essential macro- and trace elements, including P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, and Sr, highlighting the plant's diverse elemental profile. Notably, potassium (K) was found to be the most abundant element, exceeding the NIST 1515 reference value, indicating its crucial role in cellular


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metabolism and physiological regulation. Several trace elements like Fe, Mn, Zn, and Cu, were identified at significantly higher concentrations suggesting their important roles in antioxidant activity, enzymatic processes and potential anticancer activities. Although some elements exhibited lower concentrations compared to standard certified values, these variations can be attributed to environmental and species-specific factors. Overall, the findings provide strong evidence for the medicinal significance of *Citrus aurantifolia* and support further research into its potential therapeutic applications, particularly in anticancer studies.

**Declaration of Competing Interest**

The authors declare no conflict of interest.

**Acknowledgement**

The authors are sincerely thankful to Dr. M. Sudarshan, In-charge of Trace Elemental Laboratory, at UGC, DAE-CSR, Kolkata Centre for providing experimental facilities to carry out present research work. The authors also acknowledge the support of the Department of Physics, Kakatiya University, Telangana, for providing necessary facilities and encouragement.

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