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Trace Elemental Profiling of Anti-Dermatological Medicinal Plant *Azadirachta indica* unripen Fruits using Energy Dispersive X-Ray Fluorescence (EDXRF) Spectroscopy

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Abstract

Skin diseases remain a significant global health issue, inspiring the exploration of safe and effective plant-based therapeutics. *Azadirachta indica* (neem) has long been recognized in traditional medicine for its efficacy in treating various dermatological conditions. In the present research study, Energy Dispersive X-Ray Fluorescence (EDXRF) was employed to analyze the elemental composition of neem fruits, with particular focus on elements known to contribute to skin health and the results were validated using standard certified values of NIST 1515 apple leaf. The analysis revealed the presence of essential elements such as calcium (Ca: 19100.75±226.96 ppm), potassium (K: 8175.32±681.12 ppm), iron (Fe: 130.21±7.21 ppm), zinc (Zn: 9.47±4.54 ppm), manganese (Mn: 20.65±3.92 ppm), and copper (Cu: 3.99±1.01 ppm), which play important roles in wound healing, collagen synthesis, enzymatic activity, and antioxidant defense. Among these, the high concentrations of Ca and K support cellular regeneration and skin barrier function, while Fe, Zn and Mn are contributed to enzymatic activity, tissue repair, immune response and anti-oxidant defense. The findings suggest that, the therapeutic effectiveness of neem in treating skin diseases is not only due to its bioactive compounds but also strongly associated with its rich elemental profile. Overall, this study highlights the importance of trace elements in medicinal plants and confirms EDXRF as an efficient tool for rapid and accurate elemental analysis of plant-based therapeutic resources.

Keywords: Skin diseases**,** EDXRF spectroscopy, A. *indica* unripen fruits, Trace elemental analysis.

1. Introduction

Skin diseases constitute a significant global public health burden people face today, affecting individuals of all age groups and socioeconomic backgrounds. According to the World Health Organization (WHO), nearly 900 million people worldwide are living with some form of skin or subcutaneous condition at any given time (WHO, 2022). These conditions range from mild infections and inflammatory disorders to severe chronic diseases such as psoriasis, eczema, and skin cancers (Pezzolo et al., 2020). Globally, skin diseases contribute substantially to disability-adjusted life years (DALYs), reflecting their long-term impact on quality of life. In developing countries, including India, the prevalence of skin diseases is particularly high due to factors such as poor hygiene, environmental pollution, microbial infections, malnutrition, and limited access to healthcare facilities (Xi et al., 2025). Fungal infections, dermatitis, acne, and scabies are among the most commonly reported dermatological conditions in India, with millions of new cases recorded annually. Although mortality directly attributed to skin diseases is relatively low compared to other non-communicable diseases, certain conditions such as melanoma and severe infections can be life-threatening if untreated, contributing to a measurable number of deaths globally (Satish et al., 2022).

The etiology of skin diseases is multifactorial, involving genetic predisposition, microbial infections, immune dysregulation, environmental exposure, and nutritional imbalances (Prescott et al., 2017). Deficiencies or excesses of essential nutrients, including trace elements, play a critical role in the onset and progression of various dermatological disorders (Bjorklund et al., 2025). Conventional treatments for skin diseases primarily involve the use of topical and systemic medications such as corticosteroids, antifungal agents, antibiotics, and immunosuppressants (Mijaljica et al., 2022). While these treatments can be effective, they are often associated with adverse side effects, high costs, and the development of drug resistance. Consequently, there is an increasing global interest in alternative and complementary approaches, particularly those based on medicinal plants, which are considered safer, cost-effective, and culturally acceptable.

Medicinal plants have been an integral part of traditional healthcare systems for centuries and continue to play a vital role in primary healthcare, especially in developing countries. The WHO estimates that approximately 80% of the world's population relies on traditional medicine, predominantly plant-based remedies, for their healthcare needs (Jyothsna et al., 2022). These plants are rich sources of bioactive compounds such as alkaloids, flavonoids, terpenoids, and phenolic compounds, which exhibit a wide range of pharmacological activities (Velu et al., 2018). In addition to organic phytoconstituents, medicinal plants also contain essential macro- and micro-elements that significantly contribute to their therapeutic efficacy. The synergistic interaction between these organic and inorganic components enhances the overall biological activity of plant-based medicines (Vaou et al., 2022).

Among the numerous medicinal plants, *A. indica* commonly known as a neem, holds a prominent place due to its extensive therapeutic properties. Native to the Indian subcontinent, neem has been widely used in traditional systems of medicine such as Ayurveda, Siddha, and Unani for the treatment of various ailments, particularly skin diseases (Devi et al., 2023). Almost every part of the neem tree, including leaves, bark, seeds, and fruits, possesses medicinal value. Neem fruits, in particular, are known to contain a diverse array of bioactive compounds, including limonoids, fatty acids, and antioxidants, which contribute to their antimicrobial, anti-inflammatory, antifungal, and wound-healing properties (Asghar et al., 2022). These attributes make neem fruits especially relevant in the management of dermatological conditions.

While considerable research has focused on the organic constituents of *A. indica*, relatively less attention has been given to its elemental composition and the role of trace elements in its pharmacological activity. Trace elements are indispensable for human health, playing crucial roles in various physiological and biochemical processes. Elements such as zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), calcium (Ca), and potassium (K) are involved in enzymatic reactions, immune function, antioxidant defense, and maintenance of skin structure and integrity (Skalnaya et al., 2018; Prashanth et al., 2015). For instance, zinc is essential for DNA synthesis, cell proliferation, and wound healing, and its deficiency is associated with delayed healing, dermatitis, and increased susceptibility to infections. Similarly, copper plays a vital role in collagen synthesis and skin pigmentation, while iron is necessary for oxygen transport and cellular metabolism.

The imbalance of trace elements-either deficiency or excess-can lead to various health complications, including skin disorders. Zn deficiency can result in conditions such as acrodermatitis and impaired wound healing, whereas excessive zinc intake may interfere with copper absorption and immune function. Iron deficiency is commonly associated with pallor and reduced skin vitality, while iron overload can lead to oxidative stress and tissue damage (Fatimaet al., 2026). Copper deficiency may cause skin depigmentation and weakened connective tissue, whereas excess copper can contribute to toxicity and inflammation. Manganese and calcium also play important roles in maintaining skin health, and their imbalance can disrupt normal physiological processes (Jyothsna et al., 2022). Therefore, understanding the elemental composition of medicinal plants is essential not only for evaluating their therapeutic potential but also for ensuring their safety and efficacy.

In this context, advanced analytical techniques are required for accurate and reliable determination of elemental composition in plant materials. Energy Dispersive X-Ray Fluorescence (EDXRF) spectroscopy as a powerful tool for multi-elemental analysis due to its non-destructive nature, minimal sample preparation, rapid analysis, and high sensitivity. EDXRF enables simultaneous detection of a wide range of elements, from major to trace levels, making it particularly suitable for the analysis of medicinal plants. Furthermore, the technique preserves the integrity of the sample and reduces the risk of contamination, thereby ensuring accurate results (Jyothsna et al., 2020; Jyothsna et al., 2021).

The present study aims to perform trace elemental profiling of *A. indica*fruits using EDXRF spectroscopy, with a specific focus on their relevance to dermatological applications. By identifying and quantifying essential elements present in neem fruits, the study seeks to establish a scientific basis for their traditional use in the treatment of skin diseases. Additionally, the research highlights the importance of trace elements in enhancing the pharmacological properties of medicinal plants and underscores the need for comprehensive elemental analysis in phyto-therapeutic studies.

Overall, this investigation contributes to the growing field of medicinal plant research by integrating traditional knowledge with modern analytical techniques. The findings are expected to provide valuable insights into the role of elemental composition in the therapeutic efficacy of *A. indica* fruits and support their potential application in the development of safe and effective treatments for skin diseases.

2. Materials and Methods

2.1 Plant Material Collection and Preparation

Unripe fruits of *A. indica* (Neem) were collected from healthy, disease-free trees located in [insert location, e.g., Warangal, Telangana, India] during the early fruiting season. The plant material was authenticated by a qualified taxonomist from the Department of Botany, Kakatiya University, Warangal. The collected fruits were thoroughly washed with running tap water to remove adhering dust, soil particles, and other contaminants, followed by rinsing with distilled water. The cleaned samples were then air-dried under shade at ambient temperature (25–30 °C) for 10–15 days to prevent degradation of thermolabile constituents. After complete drying, the samples were further oven-dried at 50–60 °C for 24 hours to remove residual moisture.

The dried fruits were finely powdered using a stainless-steel mechanical grinder to avoid metal contamination. The powdered samples were sieved through a fine mesh (typically 100–200 µm) to ensure uniform particle size, which is essential for accurate X-ray fluorescence analysis.

Figure 1. Image of *A.indica* (Neem) unripe fruits

2.2 EDXRF Instrumentation

The elemental analysis of *A. indica* unripe fruit samples was carried out using an Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer at UGC, CSR-DAE Kolkata center (Jyothsna et al., 2020).

3. Results and Discussion

The elemental composition of *A. indica* unripe fruit samples was quantitatively analyzed using an Energy Dispersive X-ray Fluorescence (EDXRF) spectrometer, and the results are presented in Table 1. The concentrations of biologically important macro- and trace 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 validate the analytical accuracy and reliability of the technique.

The results indicate that potassium (K) and calcium (Ca) are the predominant macro-elements in the unripe fruit samples, with concentrations of 8175.32 ± 681.12 mg/kg and 19100.75 ± 226.96 mg/kg, respectively. Although these values are lower than the NIST standard for K, the Ca concentration is comparatively higher than the certified value, suggesting a rich calcium content in *A. indica*, which may contribute to its therapeutic significance, particularly in skin health and tissue repair.

Table 1. The Average Elemental Concentrations in *A. indica* unripe fruits compared with standard NIST 1515 apple leaf

Elements	*Azadirachta indica* unripe fruits	NIST 1515 apple leaf standard certified values
P	524.44±423.46	1590
S	1153.06±117.75	1800
Cl	1850.32±199.7	579
K	8175.32±681.12	16100
Ca	19100.75±226.96	15260
Mn	20.65±3.92	54
Fe	130.21±7.21	83
Cu	3.99±1.01	5.64
Zn	9.47±4.54	12.50
Se	0.79±0.24	0.05
Br	20.64±0.3	1.80
Rb	11.57±0.4	10.20
Sr	185.10±9.8	25

Among the trace elements, iron (Fe) was found at a relatively higher concentration (130.21 ± 7.21 mg/kg) compared to the standard value (83 mg/kg), indicating its potential role in enzymatic activities and oxidative stress regulation. Similarly, elements such as selenium (Se), bromine (Br), and strontium (Sr) exhibited significantly elevated concentrations compared to NIST values. The selenium (Se) (0.79 ± 0.24 mg/kg vs. 0.05 mg/kg) is an essential antioxidant element, which may enhance the dermatological efficacy of the plant through free radical scavenging mechanisms (Pincemail et al., 2022). Chlorine (Cl) and sulfur (S) were also present in appreciable amounts (1850.32 ± 199.7 mg/kg and 1153.06 ± 117.75 mg/kg, respectively). The elevated chlorine content compared to the standard suggests possible environmental or soil-based influences, while sulfur plays a crucial role in protein synthesis and antimicrobial activity, which supports the plant's traditional use in treating skin disorders (Krochmal-Marczak et al., 2025).

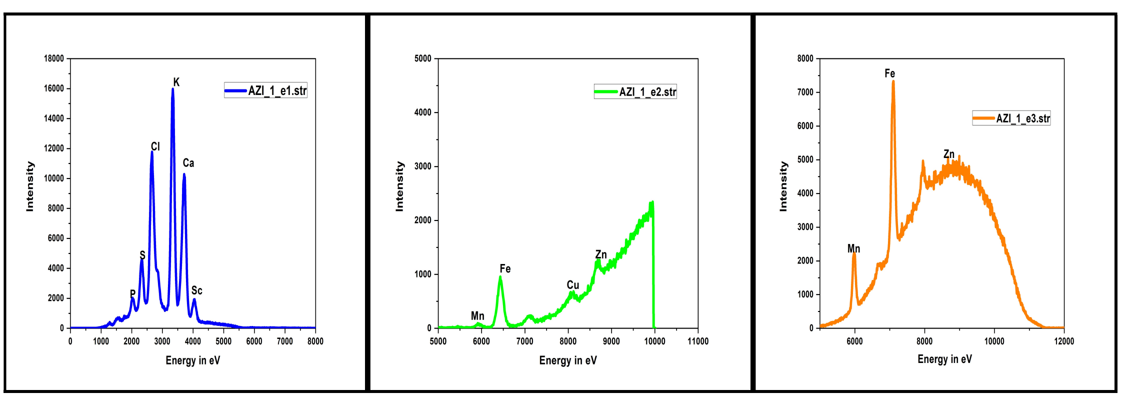

Figure 2. EDXRF spectrums of A. Indica plant sample at various energy ranges (AZI_1_e1.str, AZI_1_e2.str and AZI_1_e3.str)

On the other hand, elements such as phosphorus (P), manganese (Mn), copper (Cu), and zinc (Zn) were found to be lower than the certified reference values. However, their presence, even at lower concentrations, is significant due to their involvement in various biochemical and enzymatic processes (Hänsch et al., 2009). For instance, zinc and copper are well-known for their roles in wound healing and skin regeneration. Rubidium (Rb) and strontium (Sr), though not primary biological elements, were detected in measurable amounts (11.57 ± 0.4 mg/kg and 185.10 ± 9.8 mg/kg, respectively). The concentration of Sr was notably higher than the reference value, which may be attributed to geochemical variations in the soil where the plant was grown.

Collectively, the comparison with NIST 1515 apple leaf standard demonstrates reasonable agreement for several elements, confirming the reliability and precision of the EDXRF technique. The observed variations may be attributed to differences in plant species, environmental conditions, soil composition, and matrix effects inherent to biological samples. The presence of essential macro- and trace elements, particularly Ca, Fe, Se, and Zn, highlights the potential of *A. indica* unripe fruits as a valuable source of bioactive elements with significant dermatological and medicinal applications.

4. Conclusion

The present study used EDXRF spectroscopy to estimate the trace elemental composition of *A. indica* unripe fruits in a simple and reliable way. The results showed that the fruits contain a wide range of important elements including P, S, Cl, K, Ca, Mn, Fe, Cu, Zn, Se, Br, Rb, and Sr, reflecting their rich natural composition. Some elements, particularly Ca, Fe, Se, Br, and Sr, were found in higher amounts compared to the NIST 1515 apple leaf, which may be influenced by soil conditions and environmental factors. These elements are especially important because they support essential body functions like tissue repair, enzyme activity, and protection against oxidative stress. The presence of selenium, known for its antioxidant properties, further adds to the medicinal value of neem, especially in skin-related treatments. Although elements like P, K, Mn, Cu, and Zn were present in slightly lower amounts than the standard, they still play important roles in maintaining normal biological functions. The differences observed between the sample and the standard are natural and can be explained by variations in plant type, soil, and growing conditions. Overall, the comparison confirms that EDXRF is a dependable technique for quick and non-destructive elemental analysis, and the findings highlight that neem fruits are a valuable natural source of beneficial elements that may support their traditional use in managing skin disorders.

5. Future study

This study provides a scientific basis for further pharmacological and nutraceutical investigations, encouraging the exploration of *A. indica* as a potential candidate for the development of natural dermatological formulations. In addition, experimental and clinical studies, along with the development of standardized neem-based formulations, can enhance its application in modern dermatological treatments.

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