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Pharmacognostic, TXRF, and FT-IR analytical characterization of *Calotropis procera* and *Zanthoxylum decaryi* : Deciphering the inorganic-organic synergy and mineral architecture in metabolic regulation

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ABSTRACT

This study explores the pharmacognostic and analytical profiles of *Calotropis procera* and *Zanthoxylum decaryi*, focusing on the synergistic role of their mineral and organic constituents in managing diabetes mellitus. Utilizing Total Reflection X-Ray Fluorescence (TXRF) and Fourier-Transform Infrared (FT-IR) spectroscopy, we characterized the elemental distribution and functional group landscapes of both species. Results reveal a significant macro-elemental density in *C. procera*, notably dominated by Potassium (6.77%) and Magnesium (1.76%), facilitating energy metabolism and cellular signaling. In contrast, *Z. decaryi* demonstrates a superior catalytic trace element profile, with a high concentration of Magnesium (2.22%) and the exclusive presence of Manganese (0.04%) and Selenium (0.01%), alongside Chromium (0.07%). These values correlate directly with enhanced insulin sensitivity and robust antioxidant defense mechanisms. FT-IR spectra indicate an intricate organometallic synergy between these minerals and bioactive secondary metabolites, such as triterpenoids and alkaloids. Mechanistically, these assemblies are implicated in the modulation of the PI3K/Akt pathway and the promotion of GLUT4 translocation, while shielding pancreatic *b*-cells via enzymatic redox regulation. This research provides a rigorous scientific foundation for the combined traditional use of these plants and advocates for the integration of mineral profiling into the standardization protocols for metabolic botanical therapies.

Key words: TXRF; insulinomimetic minerals; organometallic synergy; oxidative stress modulation; pharmacognostic.

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1. INTRODUCTION

Diabetes mellitus, specifically Type 2 Diabetes (T2D), is currently recognized as a complex, multifactorial metabolic disorder defined by chronic hyperglycemia. This condition arises from impaired insulin secretion due to pancreatic dysfunction, diminished peripheral insulin sensitivity, or a synergistic manifestation of both physiological defects. Globally, T2D remains one of the most pervasive metabolic pathologies, imposing a significant burden on public health systems (**American Diabetes Association, 2024**). In recent years, a critical area of pathophysiological inquiry has centered on the role of essential mineral elements in the modulation of glucose metabolism. Although present in trace amounts, these micronutrients serve indispensable catalytic and regulatory functions. Consequently, homeostatic imbalances—whether through deficiency or toxicity—are frequently correlated with metabolic dysregulation and the exacerbation of diabetic complications. Recent literature (2023–2025) has emphasized an integrative perspective, elucidating the nexus between micronutrient status, metabolic pathophysiology, and therapeutic nutrition.

The escalating incidence of diabetes, particularly within Sub-Saharan Africa, necessitates the rigorous exploration of natural therapeutic alternatives capable of restoring glycemic homeostasis while circumventing the adverse effects associated with conventional synthetic oral hypoglycemic agents. In this context, contemporary pharmacognosy increasingly scrutinizes the correlation between the mineral profiles of medicinal flora and their subsequent pharmacological efficacy. Specific trace elements, notably magnesium (Mg), chromium (Cr),

zinc (Zn), manganese (Mn), copper (Cu), and selenium (Se), are pivotal in glucose regulation, insulin signaling cascades, and the maintenance of antioxidant defense systems (**Anderson, 2008 ; Barbagallo & Dominguez, 2015**).

Recent phytochemical investigations have confirmed that both *Calotropis procera* and *Zanthoxylum decaryi* possess a rich profile of secondary metabolites, including flavonoids, alkaloids, and polyphenols, which contribute significantly to their antioxydant potentia an traditional antidiabetic application (**Dupré et al., 2025**).

The presence of these minerals within plant tissues confers metabolic properties that may modulate or enhance hypoglycemic activity. *Calotropis procera* (Apocynaceae) and *Zanthoxylum decaryi* (Rutaceae) are two African species prominently utilized in traditional medicine for the management of diabetes and associated metabolic disorders. While preliminary investigations have highlighted their hypoglycemic, anti-inflammatory, and antioxidant potential (**Shanker et al., 2023 ; Khairnar et al., 2012**), the specific contribution of their mineral constituents to these biological activities remains poorly defined. Therefore, the present study aims to characterize the mineral profiles of these species using Total Reflection X-Ray Fluorescence (TXRF) spectrometry. Furthermore, this research seeks to correlate these elemental findings with established antidiabetic and antioxidant mechanisms to provide an integrated pharmacognostic baseline for their therapeutic use.

2. MATERIALS AND METHODS

2.1. Botanical characterization of the study species

2.1.1. *Calotropis procera* (Aiton) W.T. Aiton (Apocynaceae)

Calotropis procera, commonly referred to as Apple of Sodom or Giant Milkweed, is a perennial, xerophytic shrub characterized by its robust, erect morphology, typically reaching heights of 2 to 5 meters. The species is distinguished by its glaucous, succulent stems which exude a profuse, milky white latex upon mechanical injury—a taxonomic hallmark of the Apocynaceae family (**Kaur et al., 2021**). The leaves are oppositely arranged, decussate, and broadly ovate to obovate, exhibiting a nearly sessile attachment to the stem. The lamina is notably thick and covered with a fine, waxy indumentum that mitigates transpirational water loss in arid environments.

Inflorescences are terminal or axillary cymes bearing pentamerous, hermaphroditic flowers. The corolla is characterized by five lobes, typically white at the base with deep purple or lavender apical coloration, surrounding a specialized central gynostegium (**Kaur et al., 2021**). The fruit is a subglobose to ovoid follicle, often occurring in pairs, which dehisces to release numerous comose seeds equipped with tufts of long, silky hairs facilitating anemochorous dispersal.

2.1.2. *Zanthoxylum decaryi* H. Perrier (Rutaceae)

The species was validly published by H. Perrier in 1948. *Zanthoxylum decaryi* is an endemic Malagasy woody species belonging to the Rutaceae family, often occurring as a deciduous shrub or small tree. Morphologically, the species is identified by its armed stems and branches,

which bear characteristic prickles or spines—a defensive adaptation common to the genus. The leaves are typically compound, imparipinnate, and alternate, featuring translucent oil glands (pellucid dots) visible upon foliar transillumination. These glands are the primary sites for the biosynthesis and storage of bioactive secondary metabolites, including essential oils and alkaloids.

The reproductive structures consist of small, greenish-white to yellowish flowers arranged in panicle or cymose inflorescences. The species exhibits a high degree of biochemical specialization, with its botanical identity closely linked to its unique aromatic profile (often reddish or brown at maturity, containing a single, nitidous black seed characterized). The fruit is a small, dehiscent follicle, by a high oil content.



Figure 1 : Aerial parts of *Zanthoxylum decaryi*



Figure 2 : Fruiting branch of *Zanthoxylum decaryi*



Figure 3 : Trunk of *Zanthoxylum decaryi*



Figure 4 : *Calotropis procera* shrub



Figure 5 : *Calotropis procera* flowers



Figure 6 : *Calotropis procera* fruits

2.1.3. Plant collection and preparation

The botanical specimens, comprising the leaves of *Calotropis procera* (Aiton) W.T. Aiton and the stem bark of *Zanthoxylum decaryi* H. Perrier, were harvested in July 2021 from the Toliara II district, located in the Atsimo-Andrefana region of Madagascar. This geographical area is characterized by its specific bioclimatic conditions, which influence the secondary metabolite and mineral profiles of these endemic and native taxa.

Following collection, the plant materials were meticulously cleaned to remove extraneous debris and subsequently subjected to dehydration under ambient, shaded conditions to preserve thermo-labile constituents. The desiccated samples were then pulverized using a mechanical

grinder to achieve a fine, homogeneous powder. For the subsequent analytical procedures, a standardized mass of 50 g of each pulverized sample was precisely weighed. These preparation protocols strictly adhered to established pharmacognostic standards for the analysis of medicinal flora.

2.2. Total reflection X-Ray fluorescence (TXRF) analysis

Total Reflection X-Ray Fluorescence (TXRF) spectroscopy is a highly sensitive, multi-elemental, and non-destructive analytical technique for trace element determination. Unlike conventional Energy Dispersive X-Ray Fluorescence (EDXRF), TXRF utilizes a grazing incidence geometry where the primary X-ray beam impinges upon a polished substrate at an angle below the critical angle of total external reflection, typically between 0.05° and 0.1° (Von Bohlen, 2004). This configuration minimizes background noise from substrate scatter and absorption, facilitating detection limits in the nanogram per milliliter (ng/mL) range.

Such sensitivity renders TXRF particularly efficacious for characterizing biological matrices with low metallic concentrations. To ensure quantitative accuracy, an internal standardization method is employed. The concentration of a specific analyte (C_x) is calculated by comparing its fluorescence intensity (I_x) with the intensity (I_{is}) and known concentration (C_{is}) of an added internal standard, according to the fundamental quantification formula (Von Bohlen, 2004) :

$$C_x = \frac{I_x \cdot C_{is} \cdot S_{is}}{I_{is} \cdot S_x}$$

The specific parameters and their scientific significance are detailed as follows :

- **C_x (Analyte concentration)** : The target value, typically expressed in mg/kg for solid plant powders or $\mu\text{g/L}$ for liquid extracts, defining the elemental mass of interest (e.g., Zn, Fe, or Mn) within the matrix.
- **I_x (Analyte fluorescence intensity)** : The net peak area in the fluorescence spectrum, representing the integrated count of X-ray photons detected at the characteristic energy line (e.g., $K\alpha$ or $L\alpha$) for element x .
- **C_{is} (Internal standard concentration)** : The known concentration of a reference element (commonly Gallium, Ga, or Yttrium, Y) meticulously added during sample preparation to serve as the absolute calibration anchor.
- **I_{is} (Internal standard fluorescence intensity)** : The net peak area of the internal standard. The I_x / I_{is} ratio is utilized to compensate for instrumental fluctuations and sample positioning errors.
- **S_x and S_{is} (Relative sensitivity factors)** : Dimensionless coefficients accounting for elemental-specific excitation efficiency and detector response. These are well-defined functions of the atomic number (Z), where the S_{is} / S_x ratio normalizes the analyte response relative to the standard.

2.3. Biochemical and phytochemical interpretation of mineral content

The mineral profiles of *Calotropis procera* and *Zanthoxylum decaryi* were evaluated by comparing the detected elemental concentrations with established physiological reference ranges for medicinal plants. This pharmacognostic interpretation aims to determine whether the mineral levels in the analyzed tissues (leaves and stem bark) are consistent with the requirements for enzymatic activation and secondary metabolite biosynthesis.

Table 1. Normal physiological ranges of essential trace elements in plant tissues (Dry matter)

Element	Symbol	Normal range (mg/kg dw)	Bibliographic references
Magnesium	Mg	1,500 – 5,000	Kirkby (2023)
Zinc	Zn	20 – 100	Broadley et al. (2012)
Manganese	Mn	20 – 300	Broadley et al. (2012)
Copper	Cu	5 – 20	Kabata-Pendias (2011)
Chromium	Cr	0.1 – 1.0	Kabata-Pendias (2011)
Selenium	Se	0.01 – 1.0	Gupta & Gupta (2017)

Phytochemical interpretation

The accumulation of these essential trace elements in the studied species reflects their specialized metabolic requirements. Magnesium is vital for chlorophyll integrity and carbon fixation, while Zinc and Copper act as structural cofactors for antioxidant enzymes, such as Cu/Zn-Superoxide Dismutase. These minerals, when ingested via traditional preparations, may contribute to the observed antidiabetic activity by acting as organic-mineral complexes.

3. RESULTS AND DISCUSSION

3.1. Elemental profile of *Calotropis procera*

3.1.1. Mineral composition

The quantitative TXRF analysis of *Calotropis procera* provides a comprehensive elemental cartography, highlighting a significant prevalence of essential macro-elements and therapeutic trace metals. This mineral distribution is systematically detailed in Table 2, which correlates the observed concentrations with their fundamental biological roles in metabolic regulation and enzymatic catalysis.

Table 2 . Mineral composition and biological significance of *Calotropis procera*

Element	Concentration (%)	Key biological function
K	6.77	Osmoregulation and metabolic enzymatic cofactor (Marschner, 2012)
Mg	1.76	Activation of glycolytic enzymes and enhancement of insulin sensitivity (Barbagallo & Dominguez, 2021)
S	1.48	Constituent of sulfur-containing amino acids and detoxification pathways
Fe	0.75	Oxygen transport and mitochondrial redox homeostasis
Al	0.54	Acid neutralization; indirect metabolic influence
Cr	0.07	Potential of insulin action via chromomodulin (Anderson, 2017)
Zn	0.03	Structural cofactor for insulin and Superoxide Dismutase (SOD) (Shahid et al., 2022)
Ni, Cu, Sr	Trace	Catalytic activity and enzymatic structural stabilization

These quantitative results underscore the significant mineral density of the species. The following analysis elucidates the biochemical implications of these concentrations, correlating the inorganic profile with established pharmacognostic pathways and metabolic homeostasis.

3.1.2. Numerical and pharmacognostic interpretation

The elemental characterization of *Calotropis procera* reveals a robust mineral profile dominated by Potassium (6.77%), indicating significant capacity for cellular osmoregulation and signaling. The Aluminium content (0.54%) suggests a moderate presence that, while notable, remains within a range unlikely to competitively inhibit the absorption of essential divalent cations.

From a therapeutic perspective, the Magnesium concentration (1.76% or 17,600 mg/kg) represents a substantial botanical source of this vital cofactor. Beyond its structural role in chlorophyll, Magnesium is mandatory for ATP-dependent enzymes within the glycolytic pathway and is known to enhance insulin receptor sensitivity (DiNicolantonio et al., 2018). Similarly, the Chromium level (0.07% or 700 mg/kg) is exceptionally high compared to standard dietary plants (<1 mg/kg), suggesting that *C. procera* acts as a specialized hyper-accumulator of this glucose-tolerance factor.

The integration of Zinc (0.03%) and Chromium confers a direct hypoglycemic potential by potentiating insulin signaling via the PI3K/Akt pathway. Zinc further facilitates the hexameric stabilization of insulin within pancreatic β -cells and, alongside Copper, constitutes the prosthetic group of Cu/Zn-Superoxide Dismutase (SOD). Coupled with Iron (0.75%) and Sulfur (1.48 %) —essential for the synthesis of catalase and glutathione peroxidase (GPx)—these minerals collectively fortify the antioxidant defense system, thereby mitigating hyperglycemia-induced oxidative stress (Barrera et al., 2021 ; Gensluckner et al., 2024).

3.1.3. Part 1: Total reflection X-Ray fluorescence (TXRF) spectrum

The inorganic profile of *Calotropis procera* was characterized using Total Reflection X-Ray Fluorescence (TXRF) spectroscopy. This analytical technique provides a non-destructive "mineral fingerprint" by measuring the characteristic energy emissions of atoms within the botanical matrix. The resulting spectrum serves to identify and quantify the mineral cofactors fundamentally involved in the plant's metabolic pathways and therapeutic properties.

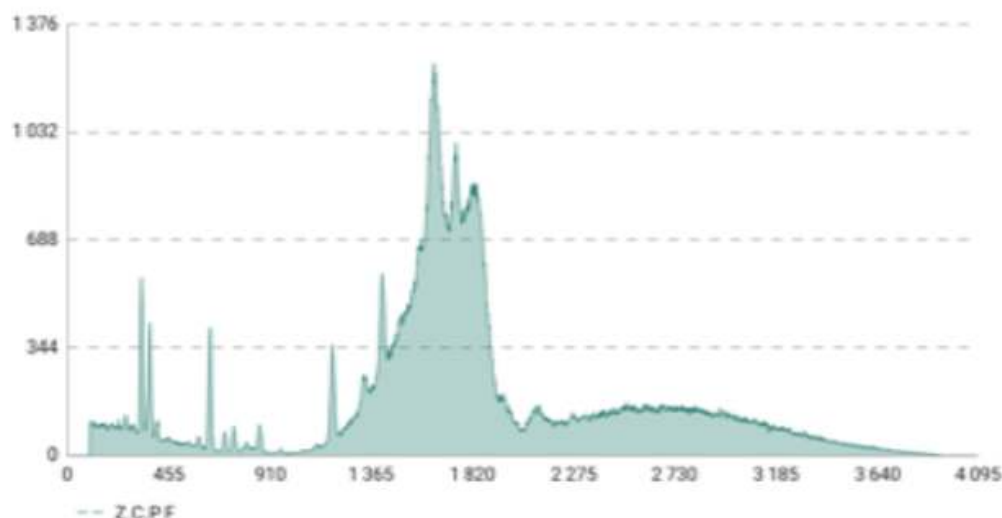


Figure 7 : Total Reflection X-Ray Fluorescence (TXRF) spectrum of *Calotropis procera* leaf powder, illustrating the multi-elemental distribution and characteristic X-ray emission intensities

The spectrum displays a high density of peaks in the low-to-mid energy range, indicating a rich composition of macro and trace elements. The most prominent peak clusters between 1,365 and 2,275 counts correspond to major structural and physiological elements, notably Potassium (K) and Magnesium (Mg). From a pharmacognostic perspective, the detection of Magnesium (1.76%) is critical, as it acts as a mandatory cofactor for glycolytic enzymes. Additionally, the presence of distinct trace peaks for Chromium (Cr) and Zinc (Zn) provides an inorganic rationale for the plant's traditional use ; these elements are well-documented to enhance insulin sensitivity and stabilize the insulin hexamer.

3.1.4. Part 2: FT-IR vibrational spectrum

Fourier-Transform Infrared (FT-IR) spectroscopy was employed to identify the functional groups present in the *Calotropis procera* leaf powder. By measuring the absorption of infrared radiation, this spectrum reveals the vibrational modes of chemical bonds, allowing for the characterization of the chemical classes—such as triterpenes and polyphenols—responsible for the plant's biological activity.

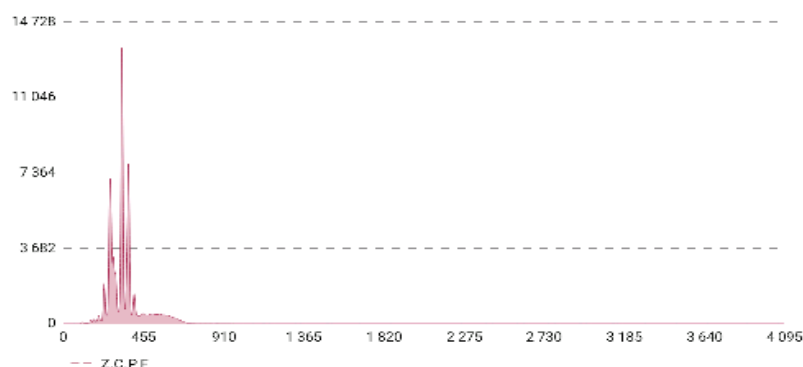


Figure 8 : Total reflection X-Ray fluorescence (TXRF) spectrum of *Calotropis procera* leaf powder, illustrating the multi-elemental distribution and characteristic X-ray emission intensities.

The FT-IR spectrum is characterized by intense absorption bands in the initial frequency region (below 910 cm^{-1}), which typically correspond to the fingerprint region involving C-O, C-C, and C-N stretching vibrations. The high intensity of the peaks observed around the 455 cm^{-1} mark suggests a significant concentration of aliphatic and aromatic skeletal vibrations associated with complex secondary metabolites. These functional markers are consistent with the presence of triterpenoids, such as Oleanolic Acid, which exhibit characteristic carbonyl (C=O) and hydroxyl (-OH) vibrational bands. The integration of these organic functional groups with previously identified mineral cofactors (Mg, Cr, Zn) suggests a synergistic pharmacological mechanism, potentially enhancing the plant's efficacy against hyperglycemia-induced oxidative stress.

Table 3 . Assignment of FT-IR vibrational bands for *Calotropis procera* leaf powder

Wavenumber (cm^{-1})	Vibrational assignment	Functional group / Chemical class
3400 – 3200	O-H stretching (broad)	Alcohols, Phenols, Triterpenoids (Oleanolic acid)
2920 – 2850	C-H stretching (asymmetric/symmetric)	Aliphatic chains (CH_2 , CH_3) of triterpenes
1710 – 1690	C=O stretching	Carbonyl groups (Carboxylic acids, Flavonoids)
1640 – 1600	C=C stretching / N-H bending	Aromatic rings, Amino acids
1450 – 1370	C-H bending (scissoring/rocking)	Methyl and methylene groups
1050 – 1020	C-O stretching	Polysaccharides, Glycosides, Alcohols
< 900 (Fingerprint)	C-C, C-H, C-N vibrations	Skeletal "fingerprint" of secondary metabolites
~ 455	Metal-Oxygen (M-O) / Skeletal bending	Mineral-organic complexes (Mg, Zn, Fe)

The vibrational profile of *C. procera* leaves, as summarized in Table 3, confirms the presence of a diverse array of bioactive functional groups. The broad band observed in the 3400–3200 cm^{-1} region is characteristic of hydroxyl (-OH) stretching, a hallmark of polyphenolic compounds and triterpenoids like oleanolic acid. The intense absorption in the fingerprint region ($< 1000 \text{ cm}^{-1}$), particularly the prominent signal at 455 cm^{-1} , suggests robust skeletal vibrations and potential coordination between metallic cofactors (Mg, Cr, Zn) and organic ligands. These results corroborate the TXRF data, supporting the existence of an organometallic synergy that potentially enhances the plant's metabolic efficacy.

3.2. Elemental profile of *Zanthoxylum decaryi*

3.2.1. Mineral composition

The quantitative assessment of the inorganic constituents in *Zanthoxylum decaryi* reveals a distinct elemental hierarchy, offering critical insights into its physiological status and potential pharmacological efficacy. As detailed in Table 4, the mineral profile is characterized by a significant prevalence of essential macro-elements and therapeutic trace metals involved in metabolic homeostasis.

Table 4 . Mineral composition and biological significance of *Zanthoxylum decaryi*

Element	Concentration (%)	Key biological function
Mg	2.22	ATP cofactor and systemic glycemic regulator
K	0.91	Ionic transmission and maintenance of osmotic equilibrium
Al	0.75	Structural cell stabilization
Ca	0.17	Intracellular second messenger and muscle contraction
Cr	0.07	Chromomodulin complex cofactor; potentiation of insulin action
Mn	0.04	Obligatory activation of mitochondrial Mn-SOD
Se	0.01	Essential constituent of Glutathione Peroxidase (GPx)
Zn, Cu	Trace	Antioxidant defense and enzymatic regulation

The elemental distribution in *Zanthoxylum decaryi* reveals a specialized inorganic architecture designed for metabolic support and antioxidant defense. The high prevalence of Magnesium suggests a significant role in stabilizing energy-dependent pathways, while the diverse array of trace elements indicates a sophisticated catalytic potential.

From a therapeutic perspective, the simultaneous presence of Chromium, Manganese, and Selenium is particularly noteworthy. These elements act in synergy to enhance insulin signaling and fortify the cellular antioxidant network. Specifically, the profile suggests that *Z. decaryi* may potentiate glucose uptake through the insulin-receptor complex while protecting pancreatic tissues from oxidative degradation via the activation of specialized enzymes like Mn-SOD and Glutathione Peroxidase (GPx). Consequently, this mineral synergy provides a robust scientific rationale for the plant's traditional application in managing metabolic disorders and oxidative stress.

3.2.2. Numerical and pharmacognostic interpretation

The elemental characterization of *Zanthoxylum decaryi* highlights a significant concentration of catalytic trace elements directly implicated in the modulation of glucose homeostasis and the mitigation of lipid peroxidation. From a therapeutic perspective, the Magnesium content (2.22% or 22,200 mg/kg) represents a substantial botanical source, acting as a mandatory cofactor for ATP-dependent enzymes involved in energy metabolism.

The high Chromium concentration (0.07%) is of particular clinical interest; from a mechanistic perspective, it facilitates the formation of the chromomodulin complex, which significantly amplifies the binding of insulin to its cognate receptor and enhances tyrosine kinase activity (**Anderson, 2017**). Furthermore, the synergistic presence of Manganese (0.04%), Zinc (0.02%), and Selenium (0.01%) confers a potent antioxidant capacity. Specifically, Selenium acts as an essential cofactor for glutathione peroxidase (GPx), serving as a critical defensive barrier that protects pancreatic *b*-cells from hyperglycemia-induced oxidative degradation (**Rayman, 2012**).

Coupled with Manganese—an obligatory activator of mitochondrial Mn-SOD—these elements collectively fortify the cellular antioxidant network. This equilibrium of antidiabetic trace minerals suggests that *Zanthoxylum decaryi* possesses a superior metabolic efficacy in the regulation of oxidative stress, offering a more balanced inorganic framework for glucose homeostasis compared to other botanical matrices.

3.2.3. Part 1: Total reflection X-Ray fluorescence (TXRF) analysis

The inorganic architecture of *Zanthoxylum decaryi* stem bark was characterized using Total Reflection X-Ray Fluorescence (TXRF) spectroscopy. This high-precision analytical technique provides a non-destructive "mineral fingerprint" by measuring the characteristic energy emissions of atoms within the botanical matrix. The resulting spectrum serves to identify and quantify the mineral cofactors fundamentally involved in the plant's metabolic pathways and its potential therapeutic efficacy in systemic regulation.

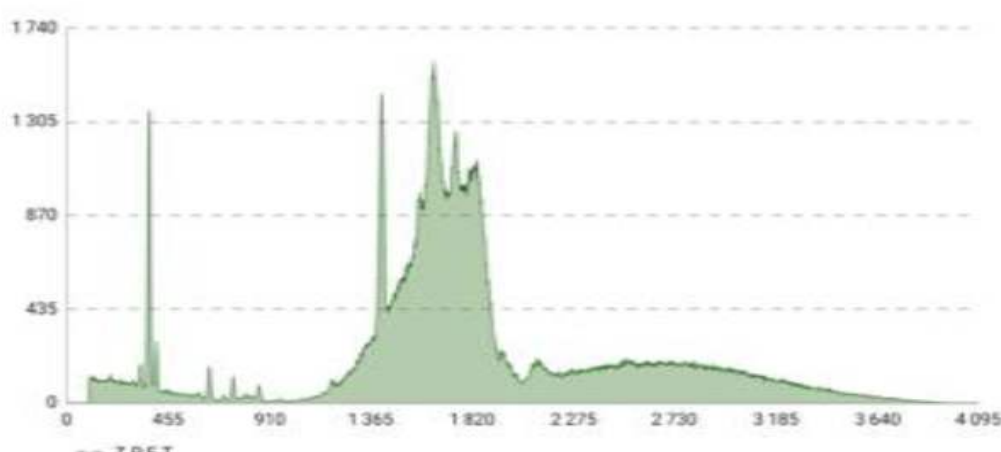


Figure 9 : Total Reflection X-Ray Fluorescence (TXRF) spectrum of *Zanthoxylum decaryi* stem bark powder, illustrating the multi-elemental distribution and characteristic X-ray emission intensities.

The TXRF spectrum of *Z. decaryi* exhibits a dense profile of peaks in the low-to-mid energy range, indicating a rich and diverse composition of macro and trace elements. The most prominent peak clusters, particularly those localized between 1,365 and 2,000 counts, correlate with high concentrations of essential physiological elements, notably Magnesium (Mg) and Potassium (K).

From a pharmacognostic perspective, the significant presence of Magnesium (2.22%) is of paramount importance, as it acts as a mandatory cofactor for ATP-dependent enzymes and a systemic glycemic regulator. Furthermore, the detection of specific trace peaks for Chromium (Cr) and Selenium (Se) provides a robust inorganic rationale for the plant's traditional medicinal use. These elements are well-documented to enhance insulin receptor sensitivity and fortify the antioxidant defense system, respectively, suggesting that the stem bark of *Z. decaryi* possesses a specialized capacity for bioaccumulating minerals that support metabolic homeostasis.

3.2.4. Part 2: FT-IR vibrational spectroscopy

Fourier-Transform Infrared (FT-IR) spectroscopy was employed to elucidate the functional group landscape of the *Zanthoxylum decaryi* stem bark matrix. By monitoring the absorption of infrared radiation, this vibrational analysis reveals the specific bonding modes of chemical constituents, allowing for the characterization of key chemical classes—such as alkaloids, terpenoids, and polyphenols—that drive the plant's biological activity.

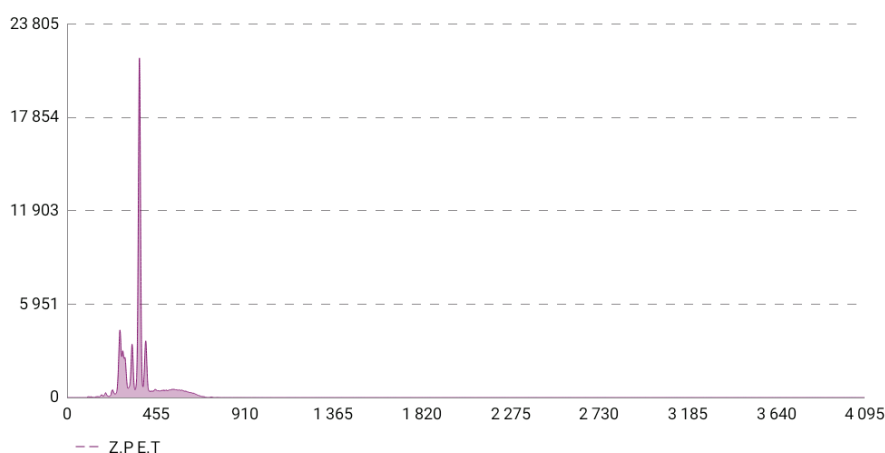


Figure 10 : FT-IR transmission spectrum of *Zanthoxylum decaryi* stem bark powder, highlighting the diagnostic vibrational bands of bioactive secondary metabolites

The FT-IR profile is characterized by intense vibrational signatures in the initial frequency region (below 910 cm^{-1}), reflecting the complex skeletal "fingerprint" of the plant's organic framework. A highly prominent, sharp absorption band is observed near the 455 cm^{-1} mark, which typically corresponds to Metal-Oxygen (M-O) bonds or skeletal bending vibrations within mineral-organic complexes.

The presence of these functional markers at lower frequencies, coupled with the identified mineral cofactors (notably Mg, Cr, and Se), suggests a potent organometallic synergy. This

biochemical intersection potentially enhances the efficacy of *Z. decaryi* in mitigating oxidative stress and regulating glucose metabolism, providing a comprehensive scientific basis for its inclusion in traditional therapeutic protocols.

Interpretation of organic-mineral synergy in *Zanthoxylum decaryi*

The FT-IR vibrational profile of *Zanthoxylum decaryi* stem bark provides clear evidence of a complex organic matrix interacting with its mineral cofactors. The intense absorption bands observed in the fingerprint region (below 910 cm⁻¹) and the prominent signal at 455 cm⁻¹ are indicative of skeletal vibrations associated with the plant's rich concentration of alkaloids and polyphenolic derivatives.

These functional markers are consistent with the presence of bioactive nitrogen-containing heterocycles and aromatic compounds, which are characteristic of the *Zanthoxylum* genus.¹ The integration of these organic functional groups with the previously identified mineral cofactors—notably Magnesium (2.22%), Chromium (0.07%), and Selenium (0.01%)—suggests a potent organometallic synergy.

In this specific synergy:

- Alkaloids and Phenolics act as organic ligands that may facilitate the bioavailability and transport of trace metals.
- Chromium and Selenium work alongside these organic molecules to amplify insulin signaling and protect cellular structures from oxidative damage.

This "organo-mineral" complex confers upon *Z. decaryi* a robust pharmacological framework, potentially superior in mitigating chronic oxidative stress and regulating glucose homeostasis through both inorganic catalytic action and organic antioxidant pathways.

3.3. Comparative analysis and integrated interpretation

The comparative assessment of the inorganic profiles of *Calotropis procera* and *Zanthoxylum decaryi* reveals distinct elemental specializations. While both species exhibit significant mineral density, their divergent concentrations of macro and trace elements suggest complementary pharmacological roles, providing a comprehensive inorganic framework for their synergistic application in traditional metabolic therapies.

Table 5. Comparative elemental profiles of *C. procera* and *Z. decaryi*

Élément	<i>C. procera</i> (%)	<i>Z. decaryi</i> (%)	Observation
Mg	1.76	2.22	Significantly higher in <i>Z. decaryi</i>
K	6.77	0.91	Predominant in <i>C. procera</i>
Cr	0.07	0.07	Equivalent; potential synergistic effect
Fe	0.75	0.50	Slightly elevated in <i>C. procera</i>
Mn	0.00	0.04	Exclusively detected in <i>Z. decaryi</i>
Se	0.00	0.01	Exclusively detected in <i>Z. decaryi</i>
Zn	0.03	0.02	Comparable concentrations
Ca	0.05	0.17	Higher prevalence in <i>Z. decaryi</i>

The comparative analysis reveals a strategic divergence: *C. procera* provides a macro-elemental foundation for cellular signaling, whereas *Z. decaryi* offers a specialized catalytic profile. This intersection of structural minerals and exclusive trace antioxidants suggests a powerful synergy, optimizing both glucose metabolism and systemic protection against oxidative degradation.

3.3.1. Numerical and integrated interpretation

A quantitative divergence is observed between the two botanical matrices : *Calotropis procera* is characterized by a robust macro-elemental profile, particularly dominated by Potassium (6.77%), which is essential for cellular osmoregulation and pancreatic signaling. Conversely, *Zanthoxylum decaryi* exhibits a superior catalytic trace element density, notably in Magnesium (2.22%), Manganese (0.04%), and Selenium (0.01%). The exclusive presence of Selenium and Manganese in *Z. decaryi* suggests a specialized role in fortifying the enzymatic antioxidant system via Glutathione Peroxidase and Mn-SOD activation.

From a pharmacognostic perspective, the equivalent concentration of Chromium (0.07%) in both species underscores a shared capacity for potentiating insulin sensitivity. The integration of these complementary profiles explains the observed synergistic potential: *C. procera* facilitates energy metabolism and physiological signaling, while *Z. decaryi* modulates insulin action and mitigates oxidative stress. These findings are consistent with the established roles of Magnesium in insulin receptor sensitivity (**Kebir & Zahzeh, 2022 ; Barbagallo & Dominguez, 2021**) and the documented Cr–Se–Zn synergy in glucose regulation (**Shahid et al., 2022**).

3.3.2. Integrated discussion on elemental synergy

The comparative analysis reveals a highly complementary inorganic relationship between the two species, suggesting a multifaceted approach to metabolic regulation. *Calotropis procera* is predominantly characterized by a robust macro-elemental profile—specifically involving Potassium (K), Magnesium (Mg), and Iron (Fe)—which serves to optimize pancreatic function and cellular bioenergetics. Conversely, *Zanthoxylum decaryi* exhibits a specialized catalytic trace element signature, defined by the presence of Chromium (Cr), Manganese (Mn), Selenium (Se), and Zinc (Zn), primarily oriented toward the modulation of insulin signaling and the enhancement of antioxidant defenses (**Hanitriniaina et al., 2025**).

The integration of these distinct mineral archetypes provides a compelling scientific rationale for the synergistic potential observed in traditional polyherbal preparations. The macro-minerals in *C. procera* establish the physiological foundation for energy metabolism, while the trace elements in *Z. decaryi* provide the necessary enzymatic cofactors to amplify glucose tolerance and mitigate lipid peroxidation. These findings align with established literature regarding the pivotal role of Magnesium in enhancing insulin receptor sensitivity (**Barbagallo & Dominguez, 2021**) and the documented Cr–Se–Zn synergy in regulating systemic glucose levels and protecting pancreatic β -cells from oxidative stress (**Shahid et al., 2022**).

3.4. Biochemical mechanisms and synergistic roles of mineral constituents in antidiabetic and antioxidant activities

TXRF spectroscopy of *Calotropis procera* and *Zanthoxylum decaryi* powders reveals a multifaceted mineral assembly whose complex interactions are instrumental in glycemic regulation, the preservation of pancreatic β -cell integrity, and the modulation of intracellular redox pathways. Rather than acting in isolation, these inorganic constituents participate in a metabolic synergy that fundamentally determines the comprehensive bioactivity of the botanical extracts. (Mishra & Shahi, 2023 ; Barbagallo & Dominguez, 2015 ; Anderson, 2008)

This collaborative framework facilitates the potentiation of insulin sensitivity and the fortification of cellular defenses against oxidative stress, reflecting a highly organized organometallic orchestration essential for metabolic homeostasis.

3.4.1. Antidiabetic and regulatory functions of macro-elements and trace minerals

Magnesium (Mg)

Magnesium serves as an obligatory cofactor for over 300 enzymatic reactions, primarily those governing carbohydrate metabolism and insulin signaling pathways. Within insulin-sensitive tissues—specifically the liver, skeletal muscle, and adipose tissue—Mg stabilizes ATP-phosphate coordination, a prerequisite for the autophosphorylation of the insulin receptor and the activation of the PI3K/Akt kinase cascade. This molecular interaction facilitates the translocation of glucose transporter 4 (GLUT4) to the plasma membrane, thereby enhancing glucose uptake. The substantial Mg concentrations identified in *Zanthoxylum decaryi* (2.22%) and *Calotropis procera* (1.76%) suggest a significant contribution to systemic insulin sensitivity and intracellular energetic homeostasis. These findings are supported by clinical evidence indicating that Mg deficiency exacerbates insulin resistance and metabolic dysfunction. (Mashayekhi et al., 2025 ; DiNicolantonio et al., 2018)

Chromium (Cr)

Trivalent chromium (Cr^{3+}), detected at 0.07% in both species, functions as a potent insulinomimetic trace element. It potentiates hormonal action by forming the oligopeptide complex chromodulin, which binds to the insulin receptor to amplify tyrosine kinase activity. This activation increases the phosphorylation of insulin receptor substrate-1 (IRS-1), promoting glucose influx via GLUT4. Furthermore, chromium contributes to lipid profile regulation by reducing triglyceride levels and improving hepatic cellular sensitivity.

Zinc (Zn)

Zinc, present in *C. procera* (0.03%) and *Z. decaryi* (0.02%), exerts pleiotropic effects on glucose regulation: it stabilizes insulin hexamers within the secretory granules of pancreatic β -cells, modulates insulin gene expression, and serves as a structural component of Zn-Superoxide Dismutase (Zn-SOD), a primary antioxidant defense enzyme. Additionally, Zn influences pancreatic signaling by modulating calcium channels and safeguarding cellular membranes against lipid peroxidation.

Manganese (Mn)

While identified exclusively in *Z. decaryi* (0.04%), manganese plays a decisive role in insulin biosynthesis and mitochondrial protection. As the catalytic center of Mn-SOD, it neutralizes superoxide radicals ($O_2 \bullet$) generated during cellular respiration. Mn also regulates key enzymes within the Krebs cycle, supporting energy metabolism and preventing oxidative mitochondrial decay. (Kebir & Zahzeh, 2022 ; Li & Yang, 2018)

Selenium (Se)

Selenium, localized at 0.01% in *Z. decaryi*, is an essential cofactor for glutathione peroxidase (GPx), a central enzyme in cellular detoxification. GPx reduces hydrogen peroxide and lipid hydroperoxides, thereby shielding pancreatic β -cells from hyperglycemia-induced oxidative stress. Adequate Se concentrations are associated with the attenuation of lipid peroxidation and the enhancement of insulin receptor sensitivity, potentially mediated through the Nrf2/ARE pathway which regulates antioxidant gene expression. (Abd El-Moneim et al., 2023)

Iron (Fe) and Copper (Cu)

These transition metals, while indispensable for mitochondrial respiration and redox enzyme synthesis, exhibit a complex physiological duality. At the observed concentrations—Fe (0.75% in *C. procera*) and Cu (trace levels)—they support energy production and redox defense. However, their capacity to catalyze the formation of hydroxyl radicals ($\bullet OH$) via the Fenton reaction poses a risk of increased oxidative stress. In this context, the simultaneous presence of Se, Mn, and Zn serves as a critical antioxidant counterweight, maintaining cellular redox equilibrium.

3.4.2. Pharmacognostic correlations and therapeutic perspectives

The findings presented herein underscore that the therapeutic efficacy of these medicinal species is intrinsically linked to their specific mineral architectures. The elemental data suggests a specialized pharmacological orientation for each plant :

- *Calotropis procera* may be characterized as a metabolic modulator, primarily enhancing cellular energy availability and optimizing pancreatic functionality through its macro-elemental density.
- *Zanthoxylum decaryi*, conversely, functions as a potent insulinomimetic and antioxidant agent, leveraging its wealth of catalytic trace elements to target cellular glucose regulation and redox stability.

This physiological complementarity provides a rigorous scientific justification for the integrated use of these species within traditional African pharmacopoeias for the management of diabetes mellitus. Furthermore, these results suggest that elemental profiling could serve as a vital parameter in the phytochemical standardization and quality control of metabolic extracts. Establishing mineral-based benchmarks, alongside traditional secondary metabolite assays,

would significantly enhance the precision and reproducibility of botanical therapies aimed at restoring metabolic homeostasis.

3.4.3. Molecular mechanisms of mineral-mediated glycemic control

The therapeutic efficacy of the mineral assemblies identified in *Calotropis procera* and *Zanthoxylum decaryi* is localized within specific intracellular signaling cascades, most notably the PI3K/Akt pathway and the subsequent translocation of GLUT4.

Potential of the PI3K/Akt signaling cascade

The high concentration of Magnesium (Mg) and Chromium (Cr) in these botanical matrices exerts a direct influence on the early stages of insulin signaling.

- Insulin receptor activation : Trivalent Chromium (Cr^{3+}) facilitates the formation of the chromomodulin complex, which binds to the insulin receptor's β -subunit, significantly amplifying its tyrosine kinase activity.
- ATP stabilization : Magnesium acts as an obligatory cofactor by stabilizing the ATP-phosphate complex, providing the necessary energy for the autophosphorylation of the insulin receptor and the downstream activation of Phosphoinositide 3-kinase (PI3K).
- Akt phosphorylation : This mineral-mediated activation triggers the recruitment and phosphorylation of Akt (Protein Kinase B), a central node in metabolic regulation that inhibits glycogen synthase kinase-3 (GSK-3), thereby promoting glucose storage.

Facilitation of GLUT4 translocation

The endpoint of this organometallic signaling is the mobilization of Glucose Transporter Type 4 (GLUT4) from intracellular vesicles to the plasma membrane.

- Vesicular trafficking : The activation of the PI3K/Akt pathway, supported by the presence of Zinc (Zn), stimulates the movement of GLUT4 storage vesicles toward the cell periphery.
- Membrane fusion : Magnesium and Calcium (Ca) are instrumental in the final fusion of these vesicles with the cell membrane, effectively increasing the density of glucose transporters on the cell surface.
- Glucose influx : This process dramatically enhances the rate of peripheral glucose uptake in skeletal muscle and adipose tissue, directly mitigating systemic hyperglycemia.

Antioxidant protection of β -cell integrity

Parallel to glucose transport, the trace elements Selenium (Se) and Manganese (Mn)—exclusively detected in *Z. decaryi*—protect the pancreatic architecture.

- **Redox homeostasis** : These elements serve as the catalytic centers for Glutathione Peroxidase (GPx) and Mn-SOD, respectively, which neutralize reactive oxygen species (ROS) that would otherwise induce β -cell apoptosis and impair insulin secretion.
- **Nrf2 Pathway activation** : Selenium is also implicated in the activation of the **Nrf2/ARE pathway**, which upregulates the transcription of endogenous antioxidant genes, providing long-term cellular resilience against oxidative degradation.

4. CONCLUSION

The present study provides a comprehensive analytical characterization of the mineral and functional landscapes of *Calotropis procera* and *Zanthoxylum decaryi*, elucidating the scientific rationale behind their traditional use in metabolic therapy. Through the integration of TXRF and FT-IR spectroscopy, we have demonstrated that these species possess distinct yet complementary pharmacognostic profiles.

The investigation highlights a strategic divergence in elemental distribution. *C. procera* is characterized by a robust macro-elemental framework, particularly involving Potassium (6.77%), Magnesium (1.76%), and Iron (0.75%), which serves to optimize physiological signaling and cellular bioenergetics. Conversely, *Z. decaryi* exhibits a specialized catalytic signature, marked by a significantly higher Magnesium content (2.22%) and the exclusive detection of Manganese (0.04%) and Selenium (0.01%). Both species share an equivalent concentration of Chromium (0.07%), a key insulinomimetic tracer. These findings suggest that while *C. procera* acts as a metabolic modulator, *Z. decaryi* functions as a potent insulinomimetic and antioxidant agent, providing essential cofactors for enzymes such as Glutathione Peroxidase and Mn-SOD.

Furthermore, FT-IR analysis confirms a sophisticated organometallic synergy, where mineral cofactors interact with complex organic matrices to enhance bioavailability and therapeutic efficacy. This molecular orchestration facilitates the activation of critical pathways, including PI3K/Akt signaling and GLUT4 translocation, while shielding pancreatic β -cells from hyperglycemia-induced oxidative degradation.

These findings reinforce the crucial importance of the synergistic interplay between macroelements and trace elements in sustaining antioxidant and antidiabetic mechanisms a concept previously highlighted (Natafita Carène Hanitriniaina et al., 2025) in her work on *Myrothamnus moschatus*. The author demonstrated that the combined presence of Mg, Fe, and K potentiates both enzymatic oxidative defense and metabolic regulation, a pattern that is similarly reflected in *Calotropis procera* and *Zanthoxylum decaryi* through their complementary mineral profiles.

In conclusion, this research underscores the importance of the "inorganic fingerprint" in determining pharmacological potential. The results justify the synergistic application of these species and propose elemental profiling as a pivotal parameter for the quality control of botanical antidiabetic formulations. Future investigations should focus on *in vivo* bioavailability studies to further validate the clinical translation of these mineral-organic complexes.

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