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Elemental profile and hydrochloric acid extractability of minerals in two indigenous varieties of *Carissa carandas* types

Ankit Kumar*, Rakesh Gehlot, Rekha Phogat, Abhishek Kumar, Kautilya Chaudhary, Ramesh Kumar

Chaudhary Charan Singh Haryana Agricultural University, Hisar, India

* e-mail: ankitnagpal1995@gmail.com

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Abstract:

This study addressed the research gap in the comprehensive elemental profiling and bio-accessibility assessment of various plant parts of two indigenous varieties of *Carissa carandas* (karonda) types.

Fresh fruits, leaves, bark, and roots were freeze-dried, ground, and sieved (300 µm). The samples underwent microwave-assisted digestion with sub-boiled nitric acid and hydrogen peroxide, followed by inductively coupled plasma mass spectrometry to quantify 20 elements. The list included major (P, K, Ca, Na, and Mg), minor (B, Mn, Fe, Cu, Zn, etc.), and toxic trace elements (Ni, Cd, Pb, etc.). The hydrochloric acid extractability was assessed using 0.03 N HCl incubation at 37°C for 4 h to simulate gastric conditions.

The study revealed significant tissue-specific variations in the mineral content: the leaf samples exhibited the highest levels of phosphorus (3,836.69 mg/kg) and magnesium (4,682.87 mg/kg), the fruits were rich in potassium (2,971.64 mg/kg), the bark accumulated high calcium amounts (8,823.55 mg/kg), and roots contained elevated sodium levels (2,129.18 mg/kg). The hydrochloric acid extractability also varied, with potassium and phosphorus showing high extractability in the fruit samples (80.06 and 87.51%, respectively) while magnesium and boron were more extractable in the leaves. Such toxic elements as lead (43.32 mg/kg, roots) and cadmium (1.22 mg/kg, bark) exceeded permissible limits, raising safety concerns.

C. carandas proved to be a potential source of essential minerals, particularly its leaves and fruits, which demonstrated good nutraceutical prospects. The study provides a scientific basis for targeted use of specific *C. carandas* plant parts with an outlook of *in-vivo* bioavailability examination.

Keywords: *Carissa carandas*, karonda, inductively coupled plasma mass spectrometry, mineral bio-accessibility, hydrochloric acid extractability, trace elements

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INTRODUCTION

Carissa carandas L., also known as karonda and Christ thorn tree, is an evergreen shrub belonging to the *Apocynaceae* family. It grows in tropical and sub-tropical regions [1]. It is native to and commonly found throughout India, Java, Sri Lanka, Malaysia, Pakistan, and Myanmar, especially in its wild form [2]. *C. carandas* thrives on inferior and marginal lands where other fruit trees fail to grow [3]. It is cultivated primarily for its colored edible fruits, but its thorns make it an excellent hedge plant for orchards. Karonda fruits are a reservoir of essential nutrients. They are exceptionally rich in iron and calcium. Therefore, karonda fruits and foods are a valuable component of anti-anemic diet. Additionally, karonda fruits contain more ascorbic acid than apples [4]. Acidic and sweet mature fruits are popular as food. Other *C. carandas* parts are also rich in various phytocompounds, i.e., saponins, phenolics, cardiac glycosides, alkaloids, flavonoids, and triterpenoids [5].

Some elements are essential for human health in small amounts because they participate in enzymatic processes. Minor and trace elements are very important due to their essential nutritious value; however, excess intake may be harmful. Therefore, an accurate analysis of these elements in food is necessary. Inductively coupled plasma mass spectrometry (ICP-MS) provides accurate quantification of minor and trace elements in food.

Previous studies offer rather meagre information about the descriptive elemental profile of *C. carandas*

fruits, leaves, bark, and roots while other berries are a popular subject of elaborated studies. For instance, Hua *et al*. [6] conducted an ICP-MS multi-element analysis on strawberries and blueberries and determined the concentration of 18 elements (B, Na, Mg, Al, P, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, Ge, As, Se, Cd, and Pb). More descriptive elemental profiles of raspberries [7, 8], wild berries [9], and other berry-type fruits have been assessed in recent years. However, these studies focus mostly on the elemental profile of berries and fail to cover other plant parts, i.e., bark, leaves, roots, stem, etc. Moreover, no comparative analysis of various plant parts with respect to elemental profile has been documented for berries, specifically for *C. carandas*.

To incorporate *C. carandas* plant into human diet, it is imperative to assess its elemental profile and hydrochloric acid (HCl) extractability. Its mineral profile may assist food scientists in developing new nutraceutical functional products.

Consequently, the goal of this research was to assess the levels of 20 elements (Li, B, Na, Mg, Al, P, K, Ca, Mn, Co, Ni, Fe, Cu, Zn, Ga, Sr, Pb, Cd, Ba, and Bi) along with their HCl extractability, i.e., bio-accessibility, in fruits, leaves, bark, and roots of two indigenous *C. carandas* types (pink and green).

STUDY OBJECTS AND METHODS

Plant material. We studied two local plant types of *Carissa carandas* L., i.e., pink and green. Fresh fruits, leaves, bark, and roots of both types were obtained from the Regional Research Station, Bawal, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. The plant parts were cleaned properly and dried in a freeze dryer to be ground into a fine powder, sieved to a uniform particle size (< 300 µm), and stored at –18°C till further experimentation.

Reagents and standards. The research featured nitric acid HNO_3 (69%; Sigma-Aldrich CHEMIE, USA), hydrogen peroxide H_2O_2 (30%) and hydrochloric acid HCl (Merck, Germany), and type I water (American Society for Testing and Materials; resistivity of 18.2 mΩ/cm at 25°C; Millipore Water Purification System).

The calibration standards for twenty elements under study (^{7}Li,^{11}B, ^{13}Na, ^{14}Mg, ^{17}Al, ^{31}P, ^{39}K, ^{40}Ca, ^{55}Mn, ^{59}Co, ^{59}Ni, ^{56}Fe, ^{64}Cu, ^{65}Zn, ^{70}Ga, ^{88}Sr, ^{107}Pb, ^{111}Cd, ^{137}Ba, and ^{209}Bi) were prepared with 0.1% HNO_3 (v/v) by adequately diluting multielement stock solutions (1,000 mg/L; Absolute Standards, Merck, Germany). Argon (purity ≥ 99.999%; Sigma Gases, New Delhi) was employed as the carrier gas.

Equipment. The multi-elemental trace analysis involved an inductively coupled plasma mass spectrometry (ICP-MS) iCAP RQ Series device (Thermo Fisher Scientific, USA) equipped with an autosampler (Autosampler ASX-560, Thermo Fisher Scientific, USA). The sample introduction system consisted of a cyclonic chamber and a concentric nebulizer. The system control relied on the Qtegra™ Instrument Control software. The samples were digested using the Anton Paar Multiwave Pro microwave digestion system. Table 1 illustrates the operational conditions.

Specification resume for ICP-MS. We placed 200 mg of each sample in microwave vials with 1 mL H_2O_2 (30%) and 4 mL sub-boiled HNO_3 (69%). The vials were left open for 20 min to prevent a sudden increase in pressure during the digestion process. The heating program consisted of three successive steps. During the first step, the temperature was rising from 25 to 100°C over 10 min. During the second step, the temperature was increasing linearly to 170°C to remain at this temperature for 30 min. The last step was a 10 min cooling phase. After opening the digestion vials, we obtained a colorless solution, clear of any particles, which signified a successful digestion. After cooling it to room temperature, we transferred the mix to 50-mL polypropylene tubes, diluted with ultrapure deionized water, and syringe-filtered it using 0.2-µm polytetrafluoroethylene membrane filters. The reagent blanks were prepared simultaneously in triplicate. The elemental analysis of the finally diluted solution in polypropylene tubes was also triplicated.

HCL extractability of minerals. We used 0.03 N HCl to extract minerals from the powdered samples. A 200-mg test sample was mixed with 10 mL of HCl (0.03 N) and incubated in an orbital shaker (100 rpm) maintained at 37°C for 4 h. The mix was then centrifuged at 5,000 rpm; the clear supernatant was dried at 100°C till 2–3 mL of extract remained. These extracts were digested in the same way as the powders. The extractable minerals in the digested samples were determined by the methods described above. To estimate the extractability, %, of each mineral component, we used the following Eq. (1):

$$\text{HCl extractablity} = \frac{\text{Mineral extractable in 0.03 N HCl}}{\text{Total mineral content}} \quad (1)$$

Table 1 Operational conditions for inductively coupled plasma mass spectrometry

Parameter	Value/setting
Nebulizer	Glass, concentric, 0.4 mL/min sample consumption
Torch vertical alignment, mm	0.2–0.5
Sampling depth, mm	15
Cooling water flow, L/min	0.59
Auxiliary gas flow rate, L/min	0.8
Dwell time, ms	50
Focus lens voltage, V	18.09
Spray chamber	Quartz, cyclonic
Radio frequency power, kW	1.54
Torch horizontal alignment, mm	0.4–1.0
Number of replicates per sample	3
Cool gas flow rate, L/min	13.9
Nebulizer gas flow rate, L/min	1.0–1.07
Sweeps/readings	10
Sample uptake rate, mL/min	0.24

RESULTS AND DISCUSSION

Carissa carandas L. is a rich source of various minerals famous for its high iron content among all fruits. In this study, we explored the mineral profiles of other karonda parts for various application prospects, i.e., nutraceuticals and pharmaceuticals. Additionally, we also assessed the hydrochloric acid (HCl) extractability of minerals to measure the bio-accessibility of these minerals in digestive systems. In Table 2, the elements are divided into major (P, K, Ca, Na, and Mg), minor (B, Mn, Fe, Cu, Zn, Li, Co, Ga, Sr, and Al), and toxic trace elements (Ni, Cd, Ba, Pb, and Bi).

Major minerals. In relation to major minerals, we observed a significant difference between various plant parts for both pink and green varieties of *C. carandas*. The highest phosphorus content belonged to the leaves of pink and green types as 3,836.69 ± 31.13 and 3,743.58 ± 111.36 mg/kg, respectively, with significant ($p < 0.05$) differences. The lowest value was observed in green karonda roots (1,810.87 ± 32.16 mg/kg). On the other hand, the HCl extractability of phosphorus was at its highest in pink karonda fruits (87.51 ± 2.60%) and at its lowest in pink karonda roots (55.55 ± 0.40%). The general trend for total phosphorus content and its extractability was leaf > bark > fruit > root and fruit > bark > leaf > root, respectively.

The high phosphorus content in leaves can be attributed to their role in photosynthesis and metabolic processes, which require significant amounts of phosphorus [10]. After absorption, phosphorus is loaded from the roots into the xylem and distributed into the shoots. It then accumulates in leaves during the plant growth phase until leaf senescence. Thereafter, plants remobilize phosphorus from the senescing leaves into the reproductive structures [11]. This could partly explain the higher extractability of phosphorus in *C. carandas* fruits. However, high phosphorus levels are not necessarily desirable, especially when it is in the form of phytate, which is not absorbed by humans and limits the bioavailability of dietary zinc and iron [12]. Moreover, high levels of phytic acid, such as in leaves and bark, restrict the solubilization of phosphorus: phytic acid binds to phosphorus and lowers its extractability [13].

Similarly, *C. carandas* leaves were rich in magnesium content with the highest content in green karonda leaves (4,682.87 ± 21.11 mg/kg) while the lowest content was observed in green karonda roots (1,217.67 ± 32.93 mg/kg). However, the extractability trends differed from those of phosphorus. The highest extractability of magnesium belonged to pink karonda leaves (97.72 ± 1.32%), followed by green karonda leaves (91.83 ± 0.99%), while the lowest was observed in green karonda roots (49.72 ± 0.96%). The general trend for both magnesium content and its extractability was the same, i.e., leaf > bark > fruit > root.

The high magnesium content in leaves can be attributed to its role as a central component of chlorophyll, which is vital for photosynthesis [14]. It facilitates the capture of light energy and the conversion of carbon dioxide and water into carbohydrates and oxygen, thereby playing a crucial role in plant growth and biomass accumulation. Therefore, most magnesium in plants is bound or incorporated in cellular compartments, with the highest concentrations in chloroplasts, i.e., leaves [12]. Beyond photosynthesis, magnesium acts as a co-factor for numerous enzymes involved in metabolic processes, including ATP synthesis, nucleic acid stabilization, and carbohydrate metabolism [15]. Leaf tissues are generally more permeable than bark or root tissues, allowing extracting agents to penetrate deeper, which might be responsible for the higher HCl extractability of magnesium [16].

Concerning potassium content, the highest values were observed in green and pink karonda fruits as 2,971.64 ± 24.08 and 2,855.44 ± 108.21 mg/kg, respectively. The lowest content belonged to pink karonda roots (789.06 ± 24.81 mg/kg). A similar trend was observed regarding the extractability of potassium. The highest potassium extractability was registered in pink karonda fruits (80.06 ± 2.67%), which was non-significantly followed by green karonda fruits (79.66 ± 3.09%). The lowest potassium extractability was observed in green karonda roots (30.34 ± 1.31%). The overall trend for both potassium content and its extractability was the same, i.e., fruit > leaf > bark > root.

Potassium plays vital roles in water relations, photosynthesis, and assimilating transport during fruit growth [17]. The present investigation aligns with the study conducted by Cheng *et al*. [18], who demonstrated that potassium accumulation varied significantly across different parts of apple trees during different growth stages. The increased potassium supply led to higher concentrations of potassium in all plant organs, supporting the notion that fruits naturally accumulate more potassium to support their development.

A study on strawberry fruit development indicated that potassium channels may play a critical role in fruit quality. The expression of potassium transporter genes was closely associated with fruit ripening and such quality attributes as firmness and sugar content. This correlation suggests higher potassium concentrations in fruits as compared to other plant parts. The high extractability can be explained by the fact that fruits possess specialized cellular mechanisms for potassium uptake and transport, which facilitate the movement of K^+ ions into vacuoles within fruit cells, increasing the solubility and bioavailability of potassium [19].

These channels are particularly active during the ripening process, allowing fruits to accumulate higher levels of potassium compared to leaves or roots. In addition, the chemical composition of fruits also affects potassium extractability. Fruits often contain organic acids and sugars that can form complexes with potassium, enhancing its solubility [20].

Unlike magnesium, phosphorus, and potassium, the calcium content was highest in green karonda bark (8,823.55 ± 310.98 mg/kg) while the lowest result belonged to green karonda fruits (958.80 ± 10.37 mg/kg).

Table 2 Elemental profile and hydrochloric acid extractability of *Carissa carandas* L. types, dry weight basis

Type	Part	Major minerals									
		Phosphorus		Potassium		Calcium		Sodium		Magnesium	
		Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %
Pink	Leaf	3,836.69 ± 31.13^{a}	72.36 ± 2.15^{d}	1,559.49 ± 61.69^{e}	76.56 ± 3.17^{a,b}	7,322.53 ± 46.20^{b}	28.17 ± 0.71^{g}	1,175.23 ± 11.65^{e}	72.87 ± 0.99^{a}	3,964.33 ± 153.66^{b}	97.72 ± 1.32^{a}
	Fruit	2,093.04 ± 67.92^{d}	87.51 ± 2.60^{a}	2,855.44 ± 108.21^{b}	80.06 ± 2.67^{a}	1,294.54 ± 47.84^{e}	77.06 ± 1.81^{a}	1,887.63 ± 81.67^{b}	66.71 ± 2.22^{b}	1,617.32 ± 58.31^{f}	85.63 ± 2.16^{c,d}
	Bark	3,073.42 ± 105.27^{b}	79.34 ± 0.79^{c}	1,229.81 ± 9.74^{d}	64.36 ± 1.61^{c}	7167.8 ± 264.90^{b}	49.82 ± 1.62^{c}	621.40 ± 1.12^{f}	68.50 ± 0.25^{b}	2,652.93 ± 114.78^{c}	83.53 ± 0.15^{d}
	Root	1,987.83 ± 60.92^{d}	55.55 ± 0.40^{f}	789.06 ± 24.81^{f}	47.39 ± 0.68^{d}	3,098.95 ± 97.77^{d}	46.36 ± 1.84^{d}	2,129.18 ± 94.04^{a}	59.05 ± 1.86^{e}	1,881.29 ± 53.25^{e}	59.23 ± 1.82^{e}
Green	Leaf	3,743.58 ± 111.36^{a}	63.31 ± 2.51^{e}	1,167.44 ± 52.62^{d}	73.77 ± 2.73^{b}	5,832.39 ± 205.03^{c}	36.72 ± 0.03^{e}	1,361.10 ± 47.85^{d}	62.70 ± 1.70^{c,d}	4,682.87 ± 21.11^{a}	91.83 ± 0.99^{b}
	Fruit	1,987.83 ± 86.01^{d}	83.90 ± 0.68^{b}	2,971.64 ± 24.08^{a}	79.66 ± 3.09^{a}	958.80 ± 10.37^{f}	68.74 ± 1.61^{b}	1,732.59 ± 29.67^{c}	65.73 ± 2.78^{bc}	1,939.45 ± 20.98^{e}	87.17 ± 2.91^{c}
	Bark	2,540.62 ± 29.77^{c}	71.28 ± 1.80^{d}	1,252.79 ± 46.95^{d}	77.05 ± 1.25^{a,b}	8,823.55 ± 310.98^{a}	33.83 ± 0.03^{f}	353.49 ± 4.46^{g}	59.46 ± 1.88^{e}	2,266.07 ± 40.85^{d}	87.29 ± 0.08^{c}
	Root	1,810.87 ± 32.16^{e}	60.36 ± 2.29^{e}	1,045.78 ± 25.47^{e}	30.34 ± 1.31^{e}	1,524.26 ± 38.47^{e}	48.27 ± 1.61^{c,d}	1,437.55 ± 47.94^{d}	61.18 ± 1.76^{de}	1,217.67 ± 32.93^{g}	49.72 ± 0.96^{f}
Type	Part	Toxic trace elements									
		Nickel	Cadmium		Barium		Lead		Bismuth		
		Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %
Pink	Leaf	–	–	0.52 ± 0.01^{c}	59.62 ± 1.83^{f}	39.28 ± 1.27^{b}	72.81 ± 1.84^{c}	7.10 ± 0.27^{d}	75.63 ± 1.23^{b}	0.18 ± 0.01^{e}	77.78 ± 3.01^{a}
	Fruit	–	–	0.36 ± 0.01^{e}	63.89 ± 1.27^{e}	8.94 ± 0.05^{f}	66.55 ± 0.60^{e}	5.41 ± 0.24^{g}	46.40 ± 0.46^{d}	0.29 ± 0.01^{d}	62.07 ± 0.17^{c}
	Bark	4.70 ± 0.06^{b}	36.81 ± 0.50^{a}	1.22 ± 0.05^{a}	84.43 ± 1.45^{a}	57.46 ± 2.28^{a}	68.78 ± 2.36^{d,e}	35.82 ± 0.23^{b}	33.58 ± 1.17^{f}	0.27 ± 0.01^{d}	48.15 ± 1.56^{e}
	Root	3.94 ± 0.03^{c}	22.84 ± 0.70^{b}	0.43 ± 0.01^{d}	83.72 ± 3.32^{a,b}	17.19 ± 0.62^{d}	87.03 ± 1.10^{a}	43.32 ± 0.47^{a}	37.49 ± 1.25^{e}	0.37 ± 0.01^{c}	56.76 ± 1.94^{d}
Green	Leaf	–	–	0.37 ± 0.02^{e}	86.49 ± 2.03^{a}	39.89 ± 0.15^{b}	77.86 ± 0.35^{b}	6.56 ± 0.01^{e}	73.48 ± 1.46^{b}	0.24 ± 0.01^{d,e}	79.17 ± 0.57^{a}
	Fruit	–	–	0.31 ± 0.01^{f}	67.74 ± 1.22^{d}	6.72 ± 0.10^{g}	70.68 ± 2.48^{c,d}	7.03 ± 0.05^{d}	37.84 ± 0.24^{e}	2.80 ± 0.11^{a}	7.86 ± 0.10^{f}
	Bark	5.29 ± 0.05^{a}	10.59 ± 0.14^{c}	0.71 ± 0.01^{b}	80.28 ± 2.53^{c}	35.78 ± 1.29^{c}	72.28 ± 3.13^{c}	17.18 ± 0.22^{c}	62.28 ± 1.85^{c}	0.25 ± 0.01^{d,e}	72.00 ± 2.40^{b}
	Root	1.49 ± 0.04^{d}	8.05 ± 0.21^{d}	0.42 ± 0.02^{d}	80.95 ± 0.51^{b,c}	15.32 ± 0.58^{e}	60.25 ± 0.22^{f}	5.90 ± 0.06^{f}	83.39 ± 2.93^{a}	0.57 ± 0.02^{b}	50.88 ± 2.25^{e}

Continuation of the Table 2

Type	Part	Minor minerals									
		Boron		Manganese		Iron		Copper		Zinc	
		Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %
Pink	Leaf	261.31 ± 6.76^{a}	83.52 ± 1.96^{b}	135.78 ± 3.06^{a}	91.58 ± 1.16^{a,b}	433.11 ± 4.68^{d}	34.02 ± 0.39^{d}	14.47 ± 0.63^{e}	74.92 ± 0.54^{ab}	127.50 ± 3.68^{d}	39.39 ± 0.46^{a}
	Fruit	44.59 ± 1.57^{d}	60.42 ± 0.49^{e}	41.94 ± 1.84^{c}	88.77 ± 2.56^{b}	151.11 ± 5.45^{f}	62.38 ± 1.12^{a}	31.36 ± 0.85^{b}	73.91 ± 3.13^{bc}	158.88 ± 5.16^{c}	36.22 ± 0.82^{b}
	Bark	58.98 ± 0.27^{c}	66.53 ± 0.84^{d}	132.30 ± 2.62^{a}	82.31 ± 2.89^{c}	2,945.25 ± 97.19^{a}	21.52 ± 0.31^{f}	39.05 ± 1.23^{a}	43.15 ± 0.08^{e}	194.29 ± 6.48^{a}	15.81 ± 0.26^{d}
	Root	14.02 ± 0.35^{f}	78.02 ± 3.16^{c}	38.68 ± 1.15^{c}	55.98 ± 1.16^{f}	830.03 ± 1.50^{c}	35.97 ± 0.91^{c}	21.09 ± 0.42^{d}	77.08 ± 1.46^{a}	155.84 ± 0.98^{c}	10.40 ± 0.22^{e}
Green	Leaf	226.67 ± 1.63^{b}	88.66 ± 2.22^{a}	132.08 ± 4.41^{a}	92.28 ± 1.33^{a}	438.24 ± 9.09^{d}	32.56 ± 1.03^{e}	16.27 ± 0.20^{e}	61.57 ± 1.11^{d}	133.29 ± 3.72^{d}	35.67 ± 0.90^{b}
	Fruit	40.11 ± 1.52^{d,e}	56.42 ± 0.92^{f}	32.65 ± 0.77^{d}	84.60 ± 0.75^{c}	112.89 ± 0.71	54.22 ± 0.49^{b}	20.65 ± 0.07^{d}	71.67 ± 0.78 ^{c}	131.18 ± 3.19^{d}	22.62 ± 1.26^{c}
	Bark	38.56 ± 1.56^{e}	60.55 ± 2.51^{e}	72.06 ± 0.19^{b}	78.22 ± 3.10^{d}	1,360.98 ± 52.75^{b}	18.43 ± 0.07^{g}	29.01 ± 0.10^{c}	60.55 ± 0.11^{d}	183.35 ± 5.95^{b}	9.26 ± 0.20^{f}
	Root	10.14 ± 0.06^{f}	50.24 ± 1.59^{g}	34.08 ± 0.18^{d}	61.13 ± 1.54^{e}	309.51 ± 7.53^{e}	34.87 ± 0.35^{c,d}	14.94 ± 0.50^{e}	75.85 ± 1.03^{a,b}	115.51 ± 3.02^{e}	15.37 ± 0.14^{d}
Type	Part	Lithium		Cobalt		Gallium		Strontium		Aluminum	
		Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %	Total, mg/kg	Extractability, %
Pink	Leaf	1.79 ± 0.04^{e}	90.50 ± 0.33^{a}	0.14 ± 0.01^{d}	–	0.13 ± 0.01^{e}	30.77 ± 0.33^{c}	291.62 ± 12.88^{c}	57.43 ± 0.83^{c}	274.85 ± 5.56^{e}	43.31 ± 0.78^{b}
	Fruit	0.62 ± 0.01^{f}	80.65 ± 2.84^{c}	–	–	0.06 ± 0.01^{f}	83.33 ± 2.70^{a}	51.69 ± 2.19^{g}	84.76 ± 3.44^{a}	130.00 ± 4.45^{f}	93.57 ± 2.24^{a}
	Bark	4.78 ± 0.13^{a}	55.44 ± 2.30^{f}	1.83 ± 0.04^{a}	14.75 ± 0.14^{b}	1.49 ± 0.06^{a}	6.71 ± 0.08^{h}	305.31 ± 1.38^{b}	49.07 ± 2.10^{d}	2,733.26 ± 34.49^{a}	8.53 ± 0.23^{g}
	Root	1.33 ± 0.01^{d}	71.43 ± 1.03^{d}	0.45 ± 0.01^{c}	–	0.32 ± 0.01^{c}	9.38 ± 0.21^{g}	188.25 ± 4.75^{e}	73.53 ± 3.25^{b}	568.66 ± 5.13^{c}	14.73 ± 0.49^{f}
Green	Leaf	1.29 ± 0.01^{d}	84.50 ± 1.75^{b}	0.13 ± 0.01^{d}	–	0.18 ± 0.01^{d}	22.22 ± 0.14^{d}	231.11 ± 2.50^{d}	55.96 ± 1.61^{c}	318.25 ± 9.24^{d}	40.09 ± 0.29^{c}
	Fruit	0.70 ± 0.01^{f}	71.43 ± 0.13^{d}	–	–	0.05 ± 0.01^{f}	80.00 ± 2.16^{b}	33.12 ± 1.02^{h}	81.22 ± 1.24^{a}	125.03 ± 5.07^{f}	94.32 ± 0.26^{a}
	Bark	2.13 ± 0.04^{b}	47.42 ± 1.45^{g}	0.81 ± 0.02^{b}	20.99 ± 0.45^{a}	0.56 ± 0.02^{b}	14.29 ± 0.03^{f}	370.33 ± 11.35^{a}	36.49 ± 0.86^{e}	1,092.93 ± 43.35^{b}	16.35 ± 0.60^{e}
	Root	1.20 ± 0.01^{e}	60.83 ± 0.88^{e}	0.10 ± 0.02^{e}	–	0.15 ± 0.01^{d,e}	20.00 ± 0.88^{e}	129.17 ± 0.08^{f}	71.40 ± 2.06^{b}	281.66 ± 0.76^{e}	34.99 ± 0.63^{d}

Data are represented as mean ± standard deviation, where n = 3. Different superscript letters ($^{a, b, c, d, e, f, g, h}$) represent significant difference across plant parts

However, the extractability showed some different scenario. The highest extractability of calcium was observed in pink karonda fruits (77.06 ± 1.81%) while the lowest was registered in pink karonda leaves (28.17 ± 0.71%). The overall trend for total calcium content and concerned extractability was bark > leaf > root > fruit and fruit > root > bark > leaf, respectively.

Calcium plays a crucial role in cambial activity and xylem development, which is important for structural integrity. Therefore, woody tissues, such as bark, tend to accumulate higher concentrations of minerals due to their slower turnover rates compared to more metabolically active tissues like leaves and fruits. This accumulation serves as a nutrient reserve that supports growth during critical developmental phases [21]. Regarding extractability, Hocking *et al*. [22] supported this notion, indicating that fruit tissues often contain more soluble forms of calcium, which are readily available for extraction.

The sodium content was at its maximum in the root samples. The highest sodium content belonged to pink karonda roots (2,129.18 ± 94.04 mg/kg) with the lowest extractability (59.05 ± 1.86%). The lowest sodium content was observed in green karonda bark (353.49 ± 4.46 mg/kg). The highest extractability belonged to pink karonda leaves (72.87 ± 0.99%). The overall trend for total sodium content and extractability was root > fruit > leaf > bark and leaf > fruit > bark > root, respectively.

The higher sodium content in roots can be attributed to its primary role in osmotic regulation, maintaining turgor pressure, and facilitating water uptake. The higher accumulation of sodium in roots indicates their direct contact with saline soils [23]. Despite the high sodium content in roots, their extractability is lower compared to leaves and fruits. This phenomenon can be explained by the compartmentalization of sodium ions within root cells, where they are sequestered in vacuoles to mitigate toxicity [24]. Compartmentalization serves as an important mechanism for plants to manage excess sodium, preventing it from interfering with cellular processes [25]. In contrast, the higher extractability in leaves indicates that these tissues may contain more soluble forms of sodium or less complex binding sites for this nutrient [26].

Minor minerals. In this study, the list of minor minerals included B, Mn, Fe, Cu, Zn, Li, Co, Ga, Sr, and Al. Leaves were found richer in total boron content as compared to other plant parts. The highest boron content was observed in pink karonda leaves (261.31 ± 6.76 mg/kg) while the lowest was detected in green karonda roots (10.14 ± 0.06 mg/kg). Extractability trends were also similar: the highest extractability of boron belonged to leaves, specifically in the pink karonda samples (88.66 ± 2.22%), while the lowest boron content was found in green karonda roots (50.24 ± 1.59%). The general trend for both total boron content and extractability was leaf > bark > fruit > root.

Boron plays a crucial role in the synthesis of pectin and maintaining cell wall structure in leaves, which is also essential for the proper functioning of chloroplasts. This phenomenon explains why boron accumulation is highest in leaves [26, 27]. It can be attributed to the presence of less complex binding sites in leaf tissues. This finding was in line with Ali *et al*. [28], who explained the high extractability in leaves as a result of physiological adaptations.

Similarly, leaves also demonstrated the highest manganese content, especially the pink karonda variety (135.78 ± 3.06 mg/kg), followed by pink karonda bark (132.30 ± 2.62 mg/kg) and green karonda leaves (132.08 ± 4.41 mg/kg). The lowest manganese content was in green karonda fruits (32.65 ± 0.77 mg/kg). We detected the highest extractability of manganese in green karonda leaves (92.28 ± 1.33%) and the lowest in pink karonda roots (55.98 ± 1.16%). The general trend for total manganese content and extractability was leaf > bark > fruit > root and leaf > fruit > bark > root.

Similar findings were reported by Imenšek *et al*. [12] in different plant parts of various elderberry (*Sambucus* spp.) interspecific hybrids. Marschner [29] observed a meagre distribution of manganese in plants: it accumulated primarily in photosynthetically active leaves. Bark was also found rich in manganese but could not be recommended for use because of its difficult accessibility and because bark harvesting is destructive for the plant [30]. Moreover, leaves showed the highest extractability of manganese as it exists in various chemical forms within plant tissues, with a significant proportion being water-soluble or bound to organic compounds [31]. Acidic environment can effectively release manganese from vacuolar storage, resulting in higher extractability from leaves. Iron is an antagonist to manganese extractability: high concentrations of iron in the plant restrict manganese mobility and solubility [32]. Plants often sequester excess manganese in vacuoles to mitigate potential damage in response to manganese toxicity. For example, Liu *et al*. [33] reported higher accumulation of manganese in peanut leaves (*Arachis hypogaea* L.) than in roots under the conditions of manganese toxicity.

C. carandas fruits are famous for their high iron content; however, its other parts are much higher in iron content with a lower HCl extractability. The highest iron content was observed for pink karonda bark (2,945.25 ± 97.19 mg/kg) while the lowest belonged to green karonda fruits (112.89 ± 0.71 mg/kg). The extractability trends were significantly different: the highest iron extractability was observed in fruit samples, specifically in the pink karonda variety (62.38 ± 1.12%), while the lowest belonged to green karonda bark (18.43 ± 0.07%). The general trend for total iron content and extractability was bark > root > leaf > fruit and fruit > root > leaf > bark, respectively.

Mohammadzadeh *et al*. [34] obtained similar results for Persian oak. A semi-systematic review of iron content across 1,228 plant species indicated that bark often contains substantial amounts of iron, although specific concentrations vary widely among species [35]. However, Imenšek *et al*. [12] detected more iron in blueberry roots than in bark while the lowest content was

observed in berries. This variation of iron across plant parts could be attributed to the lower solubility of iron and its cell compartmentalization in roots and bark [36]. The high extractability of iron in fruits may be due to organic acids, and iron is highly soluble in acidic environment [32].

The highest content of copper belonged to pink karonda bark (39.05 ± 1.23 mg/kg) while the lowest belonged to pink karonda leaves (14.47 ± 0.63 mg/kg), non-significantly followed by green karonda roots (14.94 ± 0.50 mg/kg). The highest extractability of copper was in pink karonda roots (77.08 ± 1.46%) while the lowest was in pink karonda bark (43.15 ± 0.08%). The general trend for total copper content and extractability was bark > fruit > leaf > root and root > fruit > leaf > bark, respectively.

Our studies were in agreement with Imenšek *et al*. [12] and Walkowiak-Tomczak *et al*. [37], where elderberry bark was found richer in copper than other plant parts. The higher concentration of copper in bark also indicates its structural role in plant system. However, wetland and metallophyte plant species tended to accumulate copper in roots [38, 39]. The high copper extractability from root may be traced to the ability of plants to sequester excess copper in vacuoles. It allows for the detoxification and storage of this micronutrient, which maintains cellular homeostasis and makes copper more available [40]. Additionally, copper ions can readily bind to cell walls and membranes, which can enhance the solubility of copper [41]. Similar findings were reported by Gul *et al*. [42] while working on *Berberis baluchistanica* Ahrendt: the total copper concentrations were higher in bark and leaves while the extractability was significantly greater in roots.

In a similar way, barks of both plant types contained high amounts of zinc and strontium. The highest concentration of zinc was observed in pink karonda bark (194.29 ± 6.48 mg/kg) and the lowest belonged to green karonda roots (115.51 ± 3.02 mg/kg). The extractability trends for zinc were somewhat different. We observed the highest extractability in pink karonda leaves (39.39 ± 0.46%) and the lowest in green karonda bark (9.26 ± 0.20%). The general trend for total zinc content and extractability was bark > fruit > root >leaf and leaf > fruit > root > bark, respectively.

Zinc deficiency are observed in those human populations that stick to diets high in phytate and low in meat [29]. Zinc shows good mobility within plant tissues and is actively transported through the phloem. Therefore, its content in plant roots does not exceed its content in other plant parts [43]. Zinc was preferentially available in leaves, nodes, and developing buds owing to its main role in photosynthesis and maintaining adequate levels for growth during critical developmental stages, which might be a cause for its higher extractability in leaves [44].

As mentioned previously, barks of both plant types also contained high amount of strontium: the highest was observed in green karonda bark (370.33 ± 11.35 mg/kg) and the lowest was detected in green karonda fruits (33.12 ± 1.02 mg/kg). The extractability trends for strontium showed converse results. The highest extractability belonged to pink karonda fruits (84.76 ± 3.44%) while the lowest one belonged to green karonda bark (36.49 ± 0.86%). The general trend for total strontium content and extractability was bark > leaf > root > fruit and fruit > root > leaf > bark, respectively.

The results of our study were in close agreement with those reported by Imenšek *et al.* [12], who found the highest strontium content in plant leaves followed by shoots and the lowest in flowers and fruits of various elderberry (Sambucus spp.) hybrids. Such results were most likely caused by the movement of strontium from roots to stem and then to the leaves. Strontium is poorly phloem-mobile: it is distributed in plants mainly through the xylem, which makes it poorly available in fruits [29]. In smaller quantities, strontium had a positive effect on metabolic bone diseases in humans [45]. On the other hand, strontium had various toxic effects on lungs and reproductive system when consumed in higher amounts, which majorly happens through leafy vegetables [46]. The higher extractability of strontium in fruits may be attributed to the presence of organic acids and the high acidic environment, which makes it more extractable and bioavailable in fruits [36].

In this research, the barks of both *C. carandas* types accumulated some minerals in a trace amount which was not found significant in terms of their nutritional value or toxicity to humans. Bark tended to accumulate higher concentrations of lithium with the highest content being observed in pink karonda bark (4.78 ± 0.13 mg/kg) and the lowest in pink karonda fruits (0.62 ± 0.01 mg/kg). On the other hand, the extractability was at its maximum in pink karonda leaves (90.50 ± 0.33%) and at its minimum in green karonda bark (47.42 ± 1.45%). The general trend for total lithium content and extractability was bark > leaf > root > fruit and leaf > fruit > root > bark, respectively.

This accumulation pattern was consistent with previous research suggesting that bark often serves as a reservoir for various minerals, including lithium, due to its structural and protective roles [47]. The high extractability in leaves may be attributed to their thinner cellular structure [48].

In a similar way, the highest content of gallium was observed in pink karonda bark (1.49 ± 0.06 mg/kg) while the lowest belonged to green karonda fruits (0.05 ± 0.01 mg/kg), non-significantly followed by pink karonda fruits (0.06 ± 0.01 mg/kg). The highest extractability of gallium was in pink karonda fruits (83.33 ± 2.70%) while the lowest was in pink karonda bark (6.71 ± 0.08%). The general trend for total gallium content and extractability was bark > root > leaf > fruit and fruit > leaf > root > bark, respectively.

According to Ha *et al*. [49] , the concentration of gallium in plants can vary significantly depending on the species and the specific cultivation conditions. For instance, couch grass and dandelions accumulated gallium

with concentrations ranging from 0.34 to 8.70 mg/kg in roots and from 0.21 to 6.75 mg/kg in leaves. Also, Shtangeeva [50] reported higher gallium content in roots than in leaves of natural plants. On the other hand, fruits tend to have higher extractability due to their composition, which often includes organic acids that enhance mineral solubility [32].

In our study, the bark samples of both plant types also contained meagre concentrations of cobalt and nickel. The highest cobalt content was observed in pink karonda bark (1.83 ± 0.04 mg/kg) while the lowest was observed in green karonda roots (0.10 ± 0.02 mg/kg). Cobalt was not detected in fruits. Similarly, nickel was not detected in leaves and fruits of both plant types. The highest contents of nickel belonged to green karonda bark (5.29 ± 0.05 mg/kg), which surpasses the generally accepted safe level of about 1 mg/kg established by the European Food Safety Authority [51]. The extractability of both minerals was much lower than for other minerals.

Certain plants can accumulate exceptionally high levels of nickel. For instance, *Brassicaceae* family, such as *Alyssum caricum* T.R. Dudley & Hub.-Mor. and *Thlaspi oxyceras* (Boiss.) B.D. Jacks., can accumulate up to 12.27 and 13.77 g/kg of nickel in their leaves, respectively [52], which was not a case in our study. We detected neither of the elements in fruits, which makes them safe for human consumption. While specific data on cobalt in plant parts has not been documented in research worldwide, certain plant species are known to accumulate cobalt under conditions similar to nickel. Generally, plants that thrive in metal-rich soils or those that have evolved mechanisms to tolerate heavy metals tend to accumulate both nickel and cobalt [53, 54].

Toxic trace elements. In this research, five elements were categorized as toxic, i.e., Cd, Al, Ba, Pb, and Bi. The highest content of aluminum was observed in pink karonda bark (2,733.26 ± 34.49 mg/kg) while the lowest was detected in green karonda fruits (125.03 ± 5.07 mg/kg), non-significantly followed by pink karonda fruits (130.00 ± 4.45 mg/kg). The highest extractability of aluminum belonged to green karonda fruits (94.32 ± 0.26%), non-significantly followed by pink karonda fruits (93.57 ± 2.24%). The lowest content was in pink karonda bark (8.53 ± 0.23%). The general trend for total aluminum content and extractability was bark > root > leaf > fruit and fruit > leaf > root > bark, respectively.

Aluminum is often found in higher concentrations in acidic soils, where it can be more soluble and readily taken up by plant roots [55]. Gallium behaves similarly in terms of mobility as it can accumulate in plant tissues alongside aluminum. Its higher extractability in fruits is attributed to the higher acidic conditions in fruit tissue that are responsible for its higher solubility [56].

In a similar way, the highest content of barium was observed in pink karonda bark (57.46 ± 2.28 mg/kg) while the lowest was detected in green karonda fruits (6.72 ± 0.10 mg/kg). The highest extractability of barium belonged to pink karonda roots (87.03 ± 1.10%) while the lowest was in green karonda roots (60.25 ± 0.22%).

Barium is often present in soils derived from parent materials rich in barium-bearing minerals, and its availability may depend on soil pH and organic matter content. The differences in extractability can also be attributed to the chemical forms of barium present in plant tissues. Barium can exist in various forms, including soluble salts and complexed forms that may affect its availability for uptake [57].

The highest content of bismuth was observed in green karonda fruits (2.80 ± 0.11 mg/kg) while the lowest belonged to pink karonda leaves (0.18 ± 0.01 mg/kg). Conversely, we detected the highest extractability of bismuth in green karonda leaves (79.17 ± 0.57%) and the lowest in green karonda fruits (7.86 ± 0.10%).

Bismuth is a heavy metal that can be absorbed by plants [58]. High bismuth content in green karonda fruits may indicate a more favorable interaction with soil conditions that promote bismuth uptake or an enhanced ability to tolerate and accumulate this metal. The high extractability observed in green karonda leaves suggests that its leaf tissues are more conducive to releasing bismuth into a soluble form, making it more accessible for uptake by organisms or during extraction processes [59].

The highest content of lead was found in pink karonda roots (43.32 ± 0.47 mg/kg), which was dramatically above the acceptable limit of approximately 0.1 mg/kg for foodstuffs[1]. The lowest amount belonged to pink karonda fruits (5.41 ± 0.24 mg/kg). However, the highest lead extractability belonged to the samples of green karonda roots (83.39 ± 2.93%) while the lowest belonged to pink karonda bark (33.58 ± 1.17%). The general trend for total lead content and extractability was root > bark > leaf > fruit and leaf > root > fruit > bark, respectively.

Lead is a non-essential heavy metal for plants, and its accumulation may result in various toxic effects in humans. A previous study on *Glycine max* L. (soybean) indicated substantial lead accumulation in roots compared to other plant parts [60]. While bark can store significant amounts of lead, its dense cellular structure may limit the solubility and availability of this metal for biological uptake or extraction processes [61].

We observed the highest content of cadmium in pink karonda bark (1.22 ± 0.05 mg/kg), which by far exceeded the maximal permissible levels of 0.05–0.1 mg/kg[2]. The lowest content belonged to the samples of green karonda fruits (0.31 ± 0.01 mg/kg). The highest extractability of cadmium was observed in green karonda leaves (86.49 ± 2.03%) while the lowest was detected in pink karonda leaves (59.62 ± 1.83%). The general trend for total cadmium content and extractability was bark > root > leaf > fruit and bark > root > leaf > fruit, respectively.

Cadmium is a toxic heavy metal that poses serious environmental and health risks [62]. The high extractability observed in green karonda leaves suggests that the structural characteristics of leaf tissues, including

[1] FAO/WHO. Joint FAO/WHO food standards programme codex committee on contaminants in foods. FAO/WHO. Rome, 2011.

[2] The same.

thinner cell walls and higher concentrations of organic acids, may facilitate this enhanced extractability. While bark can store significant amounts of cadmium, its dense cellular structure may limit the solubility and availability of this metal for biological uptake or extraction processes [63].

Multivariate analysis. The ***principal component analysis*** made it possible to explore the variation among different plant parts based on principal components (Fig. 1). The analysis successfully reduced the dimensionality of the dataset while preserving the most significant variance in their mineral composition and HCl extractability.

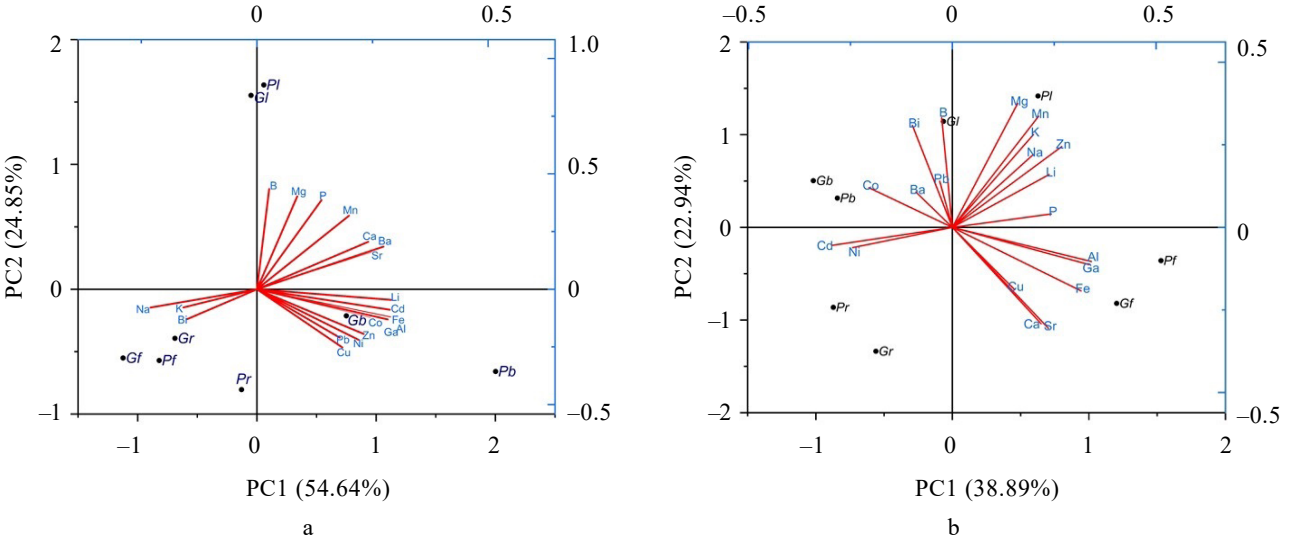
Regarding the total mineral content, the first two principal components (PCs) accounted for a significant portion of the variance, explaining 54.64 and 24.85%, respectively. Together, these two components captured 79.49% of the total variance. PC1 was affected by Mg, P, Mn, Fe, Li, Ga, Cd, Al, Ba, and Sr, which exhibited strong positive correlations with it. PC2 was primarily associated with B, K, and Na, which also showed positive correlations. In contrast, Mg, Fe, Al, and Ni had negative loadings, indicating an inverse relationship with PC2. This suggests that PC2 differentiated samples based on the relative proportions of alkali metals versus transition metals. Pink karonda bark, pink karonda roots, and green karonda bark had high PC1 scores, showing enrichment in heavy elements, while pink karonda leaves and green karonda leaves had high PC2 scores, dominated by alkali metals. The following correlations were strong, indicating geochemical relationships: Mg-P, Fe-Ga, Li-Fe, Cd-Al, and Ba-Sr. In contrast, Na-Ca, K-Sr, and Ni-Li exhibited inverse correlations.

Regarding HCl extractability, the first three principal components accounted for a major portion of the variance, explaining 38.89, 22.94, and 18.08%, respectively. Together, these three components captured 79.91% of the total variance. PC1 was strongly affected by Fe, Al, Ga, Zn, and P, while PC2 depended on B, Mn, Mg, Zn, and Na. The rest of the elements had a strong impact on PC3. Pink karonda fruits and green karonda fruits had high scores for PC1. Pink karonda leaves, pink karonda bark, and green karonda bark had high scores for PC2. Pink karonda roots and green karonda roots showed high scores for PC3. The test showed strong positive correlations between Mg-K, Mn-Zn, Fe-Ga, and Al-Ga while Cd-Ga, Co-Li, and Pb-Fe (–0.41) were found negatively correlated.

Hierarchical cluster analysis (HCA). To get a clearer understanding of the dataset, we conducted an unsupervised hierarchical cluster analysis of total mineral content and extractability. The hierarchical clustered heat map provides a visual representation of the correlation and clustering based on the intrinsic similarity between different groups (Fig. 2).

Regarding the total mineral content, we observed three major clusters. Cluster 1 included Mn, Mg, K, Na, and P, which showed high variability across samples, with significant changes in concentration. Cluster 2 included Pb, Bi, B, Ba, Co, Ni, and Cd with a low to moderate presence, which suggests their limited natural abundance or a lack of significant contamination in the samples. Cluster 3 included Fe, Ga, Al, Ca, Sr, Cu, Zn, and Li, which demonstrated moderate and uniform distribution. These elements likely play structural or metabolic roles and are uniformly present in biological and environmental samples.

Regarding mineral extractability, we also detected three major clusters. Cluster 1 included Na, K, and Bi, which showed high variability in extractability across the samples. Cluster 2 included Ni, Pb, Cu, Zn, Co, Fe, Ge, Al, Li, and Cd. These elements showed moderate



a b

Figure 1 Principal component analysis biplots for total mineral content (a) and hydrochloric acid extractability (b) of green and pink *Carissa carandas* L. types: PC1 – Principal component 1; PC2 – Principal component 2; *Pl* – pink karonda leaves; *Pf* – pink karonda fruits; *Pb* – pink karonda bark; *Pr* – pink karonda roots; *Gl* – green karonda leaves; *Gf* – green karonda fruits; *Gb* – green karonda bark; *Gr* – green karonda roots

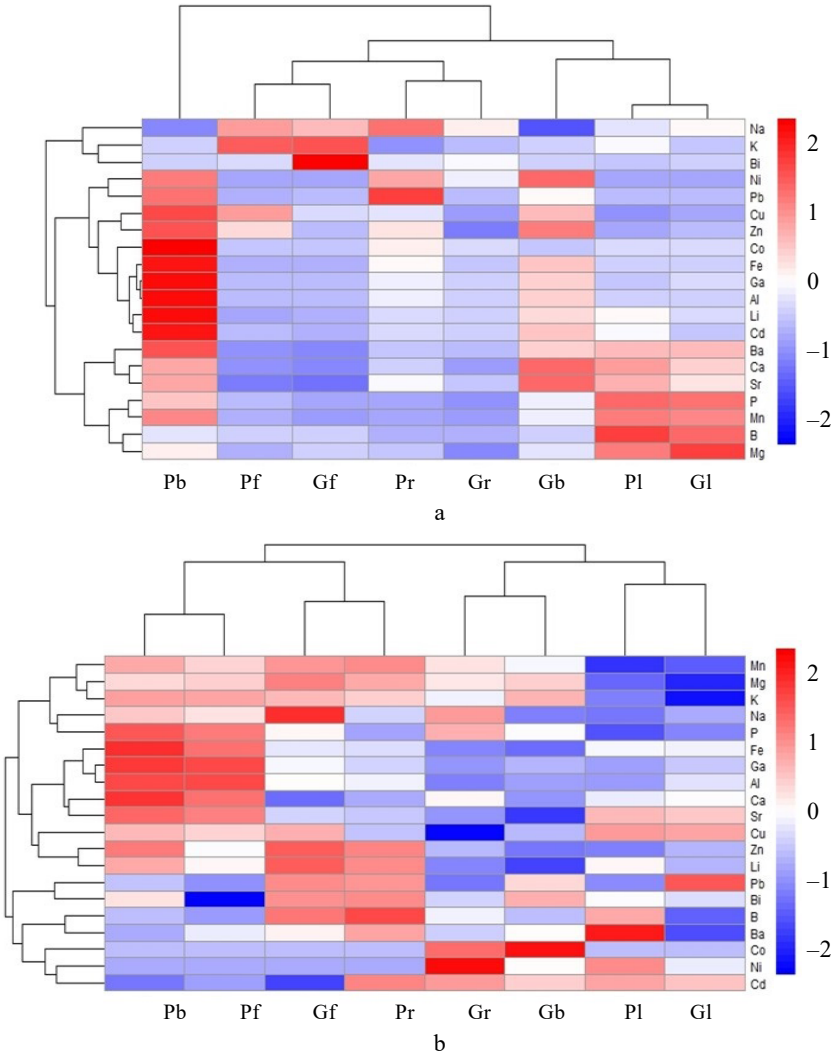


Figure 2 Non-averaged unsupervised hierarchical cluster analysis (similarity: Euclidean; linkage rule: Ward) for total mineral content (a) and hydrochloric acid extractability (b) of pink and green *Carissa carandas* L. types

and uniform extractability except for Pb. Cluster 3 included Ba, Ca, Sr, P, Mn, B, and Mg, which generally showed low to moderate extractability.

CONCLUSION

This study provided a detailed elemental analysis of different plant parts of two local types of *Carissa carandas* L. (green and pink), revealing significant variations in mineral content and hydrochloric acid extractability across tissues. Leaves were particularly rich in phosphorus and magnesium; fruits demonstrated elevated potassium levels; bark accumulated high amounts of calcium; roots showed a pronounced sodium content.

The differences in extractability suggest that the tissue-specific biochemical properties and cellular structures affected the mineral bio-accessibility, highlighting the complexity of nutrient distribution within the plant. These findings underscore the potential of *C. carandas* as a valuable source of minerals for nutraceutical and pharmaceutical applications.

Future research should focus on *in vivo* studies to further investigate the bioavailability of these minerals in human diet. Integrating advanced omics technologies, such as metabolomics and proteomics, could provide deeper insights into the underlying mechanisms that regulate mineral accumulation and extractability. Such studies will pave the way for the development of targeted functional foods and novel therapeutic agents derived from local varieties of *C. carandas*.

CONTRIBUTION

All the authors were equally involved in the manuscript and are equally responsible for any potential plagiarism.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest.

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Additional information about the authors

Ankit Kumar ORCID 0000-0003-1267-5331
Rakesh Gehlot ORCID 0000-0002-5091-4236
Rekha Phogat ORCID 0000-0003-3462-8720
Abhishek Kumar ORCID 0000-0002-4596-0668
Kautilya Chaudhary ORCID 0000-0002-9507-6491
Ramesh Kumar ORCID 0009-0007-8002-2195