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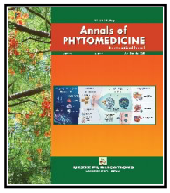

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Validation and determination of nineteen elements, including toxic heavy metals in *Prosopis juliflora* (SW.) DC. using ICP-MS

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Abstract

The toxicological and safety evaluation of herbal medicines is of prime importance as they have fewer adverse effects than other treatments. The present research focused on validating the analytical method for the determination of 19 elements, including toxic heavy metals, using ICP-MS in *Prosopis juliflora* (SW.) DC. samples collected from the Raichur District of Karnataka, known to be a hub for *P. juliflora*, a weed with medicinal properties. Samples were digested using a microwave digester. The limit of quantification (LOQ) ranged from 0.52 to 2.26 mg/kg, corresponding to a correlation coefficient of 0.99. Results showed that recovery at 0.1, 0.25, and 0.5 mg/kg spike levels ranged between 79.70-119.67%, 78.7-115.04%, and 77.28 -119.29 %, respectively, with relative standard deviation (RSD) less than 20%. The measurement uncertainty was evaluated at 0.1 mg/kg. The 19 elements, including heavy metals, were identified in *P. juliflora* leaves, bark, mature and immature pods using a validated method. *P. juliflora* had higher concentrations of manganese, rubidium, barium and copper. Conversely, the concentrations of the toxic heavy metals are below the LOQ.

1. Introduction

In India, *P. juliflora* is a significant invasive plant (Somveer *et al.,*2024). This xerophytic plant is most prevalent in the Raichur District of Karnataka, India, and can be found in semi-arid and desert areas. This plant produces pods with high nutrient content and bioactive potential (de Lemos *et al.,* 2023). Primarily belonging to the Leguminosae (Mimosaceae) family, *P. juliflora* is one of the most widely used herbal treatments for diabetes mellitus (Ukande *et al.,* 2019). In addition to its medicinal properties, it also improves soil fertility, decreases soil erosion, and provides an extra energy source and biodiesel production (Bibi *et al.,* 2023; Rajamohan *et al.,* 2022). With drooping branches and a deep root structure that grows laterally, it is a tough, drought-resistant evergreen tree (Sawal *et al.,* 2004). This tree can thrive in any type of extreme weather and soil, including alkaline soil, stony, sandy, thick clay, and even in environments without soil and water (Mohammed *et al.,* 2019; Chella Gifta *et al.,* 2022). According to studies, *P. juliflora* has a variety of medicinally useful chemical compounds that assist in inhibiting glial cell activation and drug-resistant fungi. Vitamin C can also be obtained naturally from the pods, leaves, and bark (Alagbe, 2020). Additionally, the plant's leaves and pods prevent gas generation during ruminal digestion, and its fruits have anti-lung cancer capabilities (Pramod *et al.,* 2024).

One of the main concerns in recent years has therefore been the toxicological and safety evaluation of these herbal medicines, as they have fewer adverse effects than other treatments. However, the detection of hazardous metals in various herbal preparations and herbal ingredients has raised concerns (Vinogradova *et al.,* 2023). Medicinal plants have recently caught the attention of researchers as potential remedies for heavy metal toxicity (Ceramella *et al.,* 2024; Deshwal *et al.,* 2023). However, researchers now assert that these preparations are unhealthy due to their high metal content (Sun *et al.,* 2023.) The heavy metal and elemental analysis of *P. juliflora* has not been studied, even though research on the medicinal properties, toxicological effects, and macro and micronutrient analysis of various medicinal, ayurvedic, and herbal plants has been documented in literature, reviews, and some studies (Kaushik *et al.,* 2023; Nagaraj *et al.,* 2023).

For multi-element determination at the trace level, ICP-MS provides a reliable and efficient technology with better sensitivities than traditional approaches (Kukusamude *et al.,* 2024). Method development and validation are crucial components of analytical processes, ensuring that the composition, purity, efficacy, and stability of the analysed samples are accurate, precise, and reliable (Kilic *et al.,* 2023). The purpose of this research is to develop and to validate an analytical method using ICP-MS to ascertain whether *P. juliflora* samples contain toxic and other elements. The validated analytical method could be used to generate baseline data on the simultaneous determination of 19 elements, including toxic heavy metals in *P. juliflora* collected from different locations of the Raichur District of Karnataka.

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2. Materials and Methods

2.1 Plant authentication

The plant was authenticated by Dr. C. Haleshi, Assistant Professor at Plant Taxonomy and Medicinal Plants Laboratory, Department of Studies in Botany, Davangere University, Davangere, Karnataka, India. The specimen was deposited in the Department of Studies in Botany, Herbaria Davangere University, Davangere, with Voucher Specimen Number HDUD519.

2.2 Chemicals and laboratory reagents

Concentrated trace metal grade nitric acid (69.0%-70.0%) and hydrochloric acid (36.5-38.0%) were procured from J.T. Baker, USA. Standards of cadmium, lead, arsenic, chromium, nickel, selenium, cobalt, and antimony procured from Perkin Elmer, USA, with a 1000 µg/ml concentration. Ultrapure deionized water (18 MÙ.cm), obtained from a Millipore unit, was used throughout the experiment. All the prepared solutions were stored at a room temperature of 25!.

2.3 Standard Preparation

Working standards solutions (10 mg/l) were prepared from the standard stock solutions (1000 mg/l) of each element by dilution with 2% v/v trace metal grade nitric acid in water. Calibration plots of nineteen elements were established at seven concentration levels (10, 50, 100, 250, 500, 750, and 1000 µg/l). All the measurements were carried out by the collision mode using helium as a target for polyatomic interferences. At regular intervals during analysis, a standard level of 100 µg/l was analysed to assess instrument stability. Additionally, 2% nitric acid in water was regularly run with the samples to verify the absence of carryover and cross-contamination. Sample blanks were formulated without samples following the entire process of analysis.

2.4 ICP-MS operating condition

The instrument used for the measurement of 19 metals was NexION 350 ICP-MS (PerkinElmer, USA). The NexION ICP MS with Triple cone interference with quadrupole ion detector technology provides superior analytical performance with great sensitivity and stability. Instrumental parameters and sample introduction components are shown in Table 1.

Table 1: ICP MS instrumental conditions

S. No.	Instrument parameter	Unit
1.	Nebulizer flow	0.92 ml/min
2.	Auxillary flow	1.5 ml/min
3.	Plasma gas flow	15 l/Min
4.	RF power	1500 watts
5.	Nebulizer	Meinhard glass
6.	Spray chamber	Glass cyclonic
7.	Cones	Nickel sampler and skimmer
8.	Sweeps	20
9.	Replicates	3
10.	Helium gas flow	3.3 ml/min

2.5 Method validation

The validation of the method was performed following the guidelines of Eurachem.

2.5.1 System suitability

To check the system suitability, six replicates of a specified concentration (0.1mg/kg) of 19 individual metals were injected, and the repeatability for intensity was observed.

2.5.2 Specificity

Analyzed six samples (without spiking analyte) and six LOQ spiked matrix samples of a representative commodity and reported the analytical equivalence of the blank sample concerning intensity. The percent interference must be less than or equal to 20%.

$$\text{Average intensity in blank sample} = \frac{\text{Average intensity in blanksample}}{\text{Average intensity in loqspike sample}} \times 100$$

2.5.3 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection represents the lowest analyte concentration that can be detected. The limit of quantification corresponds to the lowest analyte concentration that can be quantified. Three times and ten times the standard deviation of six separately prepared matrix blanks was considered for the determination of LOD and LOQ.

2.5.4 Accuracy and recovery

A sample solution without spiking elements was prepared in six replications and injected. Spiking at three selected concentration levels at LOQ, 5 × LOQ, and 10 × LOQ, the analytical quality of results was ensured by the percent recovery studies for the targeted 19 elements.

2.5.5 Precision

Precision is expressed as the extent of variation in the results, calculated without considering the influence of the matrix. It is typically evaluated using the relative standard deviation (RSD) from six replicate determinations of a single sample. Six preparations of *P. juliflora* were spiked with beryllium (Be), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), gallium (Ga), arsenic (Ar), rubidium (Rb), silver (Ag), cadmium (Cd), indium (In), cesium (Cs), barium (Ba), thallium (Tl), lead (Pb), bismuth (Bi), uranium (U) as per the test method, and the %RSD of all elements across the six preparations was calculated.

2.5.6 Ruggedness

When a method is being subjected to the validation process, the ruggedness parameter is strongly endorsed. Prepared six preparations of *P. juliflora* sample spiked with all 19 analytes at 0.1 mg/kg as per the test method. The study was performed by different analysts on different days using a different set of solutions.

2.6 Determination of 19 elements in *P. juliflora* leaves, bark, and pods

P. juliflora leaves, bark, and pod samples were collected from twenty different locations of the Raichur District of Karnataka and analyzed using ICP-MS to determine the range of 19 elements. The samples

were carefully rinsed with milli-Q water to remove any soil adhering to their surface. A metal-free sample homogenizer has been used to homogenize the sample. The homogenized sample was transferred into the LDPE ziplock pouch. Weighed 0.25 g samples using a 0.01 mg sensitive weighing balance and transferred them into a PFA digestion vessel. The weighed sample was added with 7 ml of suprapure nitric acid and 0.5 ml of hydrochloric acid. After allowing the vessels to stand in the fume hood at room temperature for 10-15 min., the digestion vessel was sealed and placed in the microwave digester. Two steps in the digestion procedure were used to complete the process. The vessels' temperature was raised to 180°C in 15 min. and it was maintained there for an additional 15 min. to allow full digestion. The microwave digester's vessels were taken out and stored in the fume hood when the digesting process was finished and the cooling period had passed. The vessel was carefully opened and left undisturbed until the brown fumes dissipated. The total digested sample in the liquid form was transferred to the volumetric flask. The digestion vessel was rinsed repeatedly with Milli-Q water and transferred to the volumetric flask. The final volume was adjusted to 50 ml and subsequently filtered using a 0.22 µm filter. Along with samples, a blank was prepared through all stages of preparation without a sample. The validated analytical method was used to determine the 19 elements in *P. juliflora* using ICP-MS.

3. Results

3.1 Method validation

System suitability was evaluated using the percent coefficient of variation (CV), and was within 20%, in accordance with Eurachem guidelines for all 19 elements. Sample specificity relied on the targeted elements and their potential interferences. Polyatomic ions often produce matrix and spectrum interferences due to high analyte concentrations in the sample. ICP-MS proved effective for determining mass concentrations ranging from parts per trillion to parts per million in a single analysis run. The linear regression coefficient (R^2) for calibration curves from seven standards, prepared as per the guidelines, was used to validate linearity. All 19 analyzed elements exhibited r^2 values exceeding 0.99, indicating satisfactory linearity (Table 2).

The LOD and LOQ values for individual heavy metals in *P. juliflora* samples were determined (Table 3). Sample blanks and spiked samples (0.1 mg/kg expected concentration) were tested to derive these values. The LOQs varied by product, ranging from 0.52 to 2.26 mg/kg.

Table 2: Coefficient of determination (r^2) and per cent recovery of 19 elements analyzed in *P. Juliflora* using ICP-MS

Elements	Coefficient of determination (r^2)	Spike conc. at LOQ level (mg/kg)		Spike conc. at 5 × LOQ level (mg/kg)		Spike conc.at 10 × LOQ level (mg/kg)	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Be	0.999	100.11	0.33	94.22	1.13	96.00	0.66
V	0.999	105.10	0.64	104.03	0.78	119.29	0.48
Cr	0.999	107.60	0.45	104.75	0.97	116.57	0.76
Mn	0.999	81.21	0.42	95.10	0.65	112.58	0.78
Co	0.999	106.75	0.64	111.76	0.24	112.64	0.89
Ni	0.999	99.48	0.50	95.60	0.99	96.15	0.40
Cu	0.999	90.88	0.48	98.16	0.30	98.59	0.44
Ga	0.999	94.01	0.71	93.77	1.09	93.63	0.43
As	0.999	79.70	0.32	78.70	1.27	77.28	0.38
Rb	0.999	95.55	0.66	113.9	2.41	113.56	0.41
Ag	0.999	95.84	0.36	96.64	0.33	87.89	1.07
Cd	0.999	83.65	0.60	81.02	1.15	80.68	0.43
In	0.999	113.83	0.37	111.26	0.94	112.25	1.00
Cs	0.999	115.55	0.59	115.04	1.14	115.01	0.84
Ba	0.999	115.73	0.42	114.70	0.96	114.85	0.58
Tl	0.999	112.61	0.54	106.59	0.89	109.08	0.89
Pb	0.999	109.01	0.50	102.78	0.86	104.99	0.89
Bi	0.999	97.83	0.49	92.60	0.82	94.31	0.39
U	0.999	119.67	0.33	112.09	0.98	115.41	0.79

Table 3: LOD, LOQ and ruggedness of 19 elements analyzed in *P. Juliflora* using ICP-MS.

Elements	LOD (mg/kg)	LOQ (mg/kg)	Ruggedness	
			Recovery (%)	RSD (%)
Be	0.22	0.67	114.45	0.91
V	0.45	1.38	110.35	1.03
Cr	0.32	0.98	109.80	0.53
Mn	0.74	2.26	80.07	0.67
Co	0.45	1.38	108.83	0.56
Ni	0.34	1.03	110.49	0.51
Cu	0.38	1.17	107.42	0.79
Ga	0.46	1.40	88.38	0.72
As	0.17	0.52	88.62	0.37
Rb	0.61	1.86	87.98	0.67
Ag	0.22	0.69	93.73	0.45
Cd	0.33	1.00	85.50	0.72
In	0.27	0.84	112.61	0.39
Cs	0.45	1.37	117.20	0.48
Ba	0.41	1.26	112.57	0.39
Tl	0.4	1.22	111.27	0.47
Pb	0.36	1.12	114.56	0.39
Bi	0.31	0.96	90.54	0.41
U	0.26	0.80	116.50	0.33

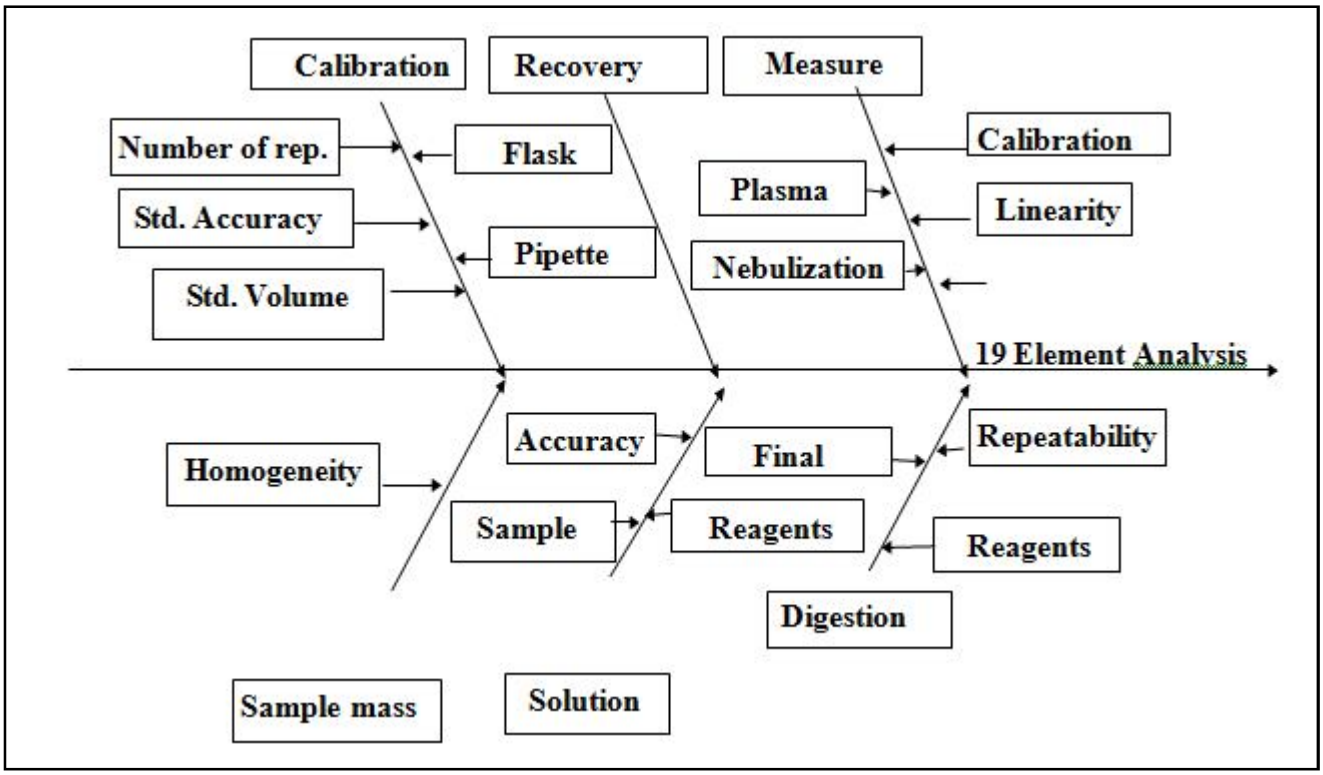

Figure 1: Fishbone diagram indicating different sources of measurement uncertainty.

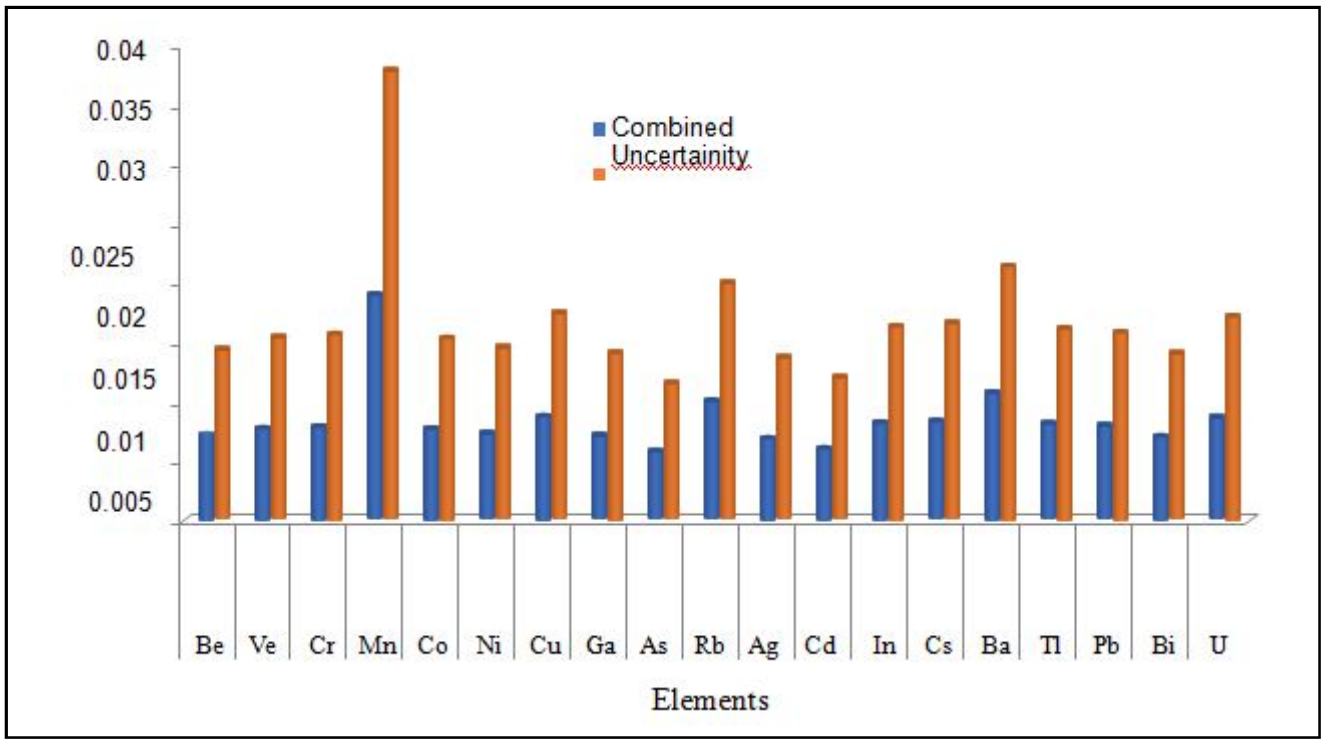

Figure 2: Combined and expanded uncertainty of 19 elements in *P. Juliflora*.

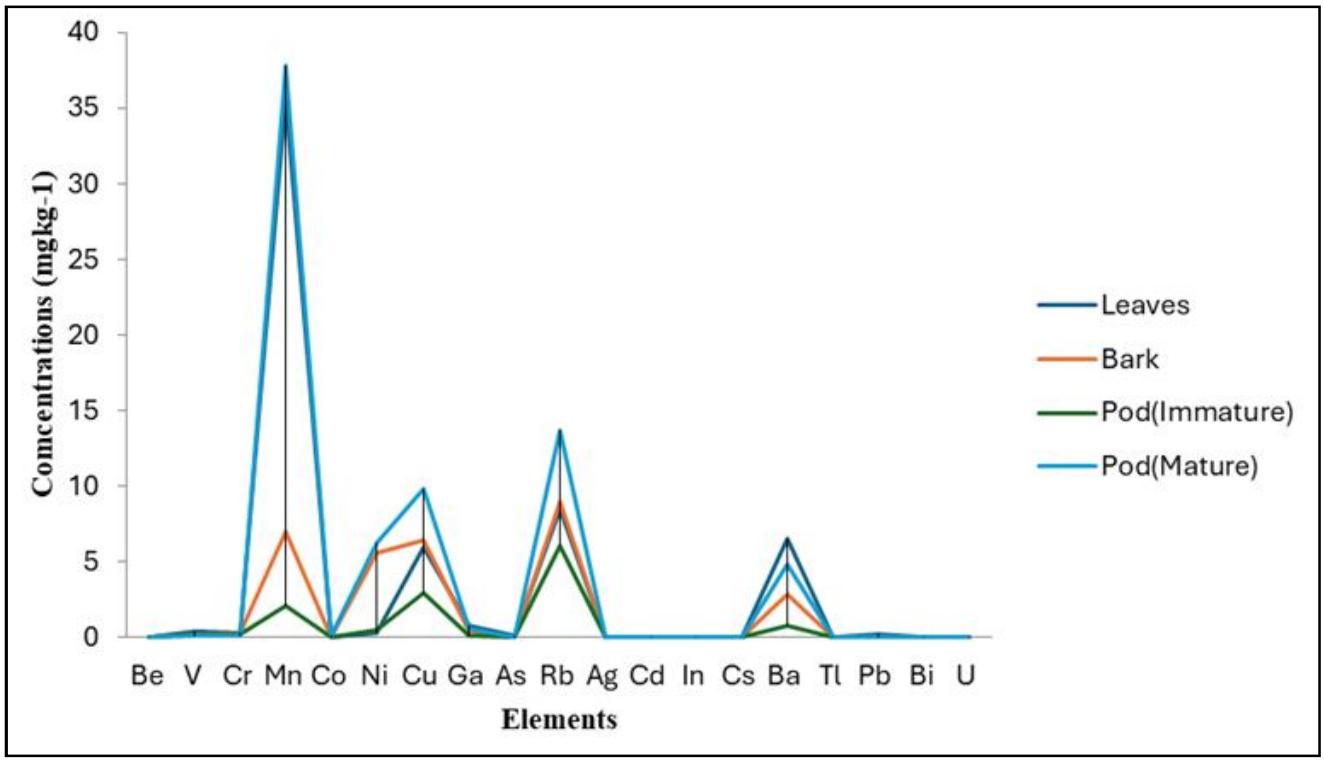

Figure 3: Concentration of 19 elements in leaves, bark, and pods of *P. juliflora.*

Ruggedness was evaluated by comparing recovery (%) obtained by two different analysts using identical microwave-assisted ICP-MS conditions. Both analysts achieved recoveries within acceptable limits (70-120%).

3.2 Measurement uncertainty

Sources of Type A and Type B uncertainty were presented in a fishbone diagram (Figure 1). Measurement uncertainty (Ux) for heavy metals at 0.1 mg/kg ranged from ± 0.01 to ± 0.04 mg/kg (Figure 2).

3.3 Simultaneous analysis of 19 elements in *P. juliflora* using ICP-MS

Quantitative analysis revealed that leaves contained 35.73 mg/kg Mn, 8.51 mg/kg Rb, 6.10 mg/kg Cu, and 6.59 mg/kg Ba. The bark contains 6.02 mg/kg Rb, 5.67 mg/kg Ni, 6.50 mg/kg Cu, and 2.91 mg/kg Ba. Mature pods contain 37.84 mg/kg Mn, 6.36 mg/kg Ni, 4.90 mg/kg Ba, and 9.94 mg/kg Cu. Immature pods contain 2.94 mg/kg Cu and 6.02 mg/kg Rb. These values are illustrated in Figure 3. Toxic elements, including Pb, Cd, As, and Cr, were found below quantification limits in all plant parts analyzed.

4. Discussion

The results indicate that *P. juliflora* is a significant source of several essential elements such as manganese (Mn), copper (Cu), and rubidium (Rb). These elements play crucial roles in human physiology: Mn is necessary for enzyme activation and bone matrix synthesis, as supported by Kippler and Oskarsson (2024) and Taskozhina *et al.* (2024). Copper (Cu), the third most abundant trace element in the human body, is involved in multiple enzymatic processes (Osredkar and Sustar, 2011). Rubidium (Rb) has been suggested to help in reducing blood pressure and mitigating the risk of cardiovascular disease (Jiang *et al.,* 2024).

A similar study by Radjassegarin *et al.* (2012) reported that, among the 13 elements investigated, K (8096.97 mg/kg), Na (581.53 mg/kg), Cu (2.06 mg/kg), Mg (678.4 mg/kg), Fe (476.93 mg/kg), Al (321.5 mg/kg), Co (0.22 mg/kg), Zn (4.46 mg/kg), Ni (0.56 mg/kg) Mn (25.77 mg/kg), Pb (13.33 mg/kg), and Cr (13.18 mg/kg) on dry weight, were present at high concentrations in *Eclipta prostrata, Adhatoda vasica, Phyllanthus amarus, Hybanthus enneaspermus, Cardiospermum halicacabum, Acacia nilotica,* and *Denolix elata*. Notably, the toxic element Cd was below detectable levels in all samples, while Cr, Pb and Ni were within the permissible limits prescribed by the World Health Organization (WHO).

Comparison with the study by Radjassegarin *et al.* (2012) shows similar patterns in elemental concentration across various medicinal plants. Their study reported high levels of essential elements, including K, Na, Cu, Fe, and Mn in species like *Eclipta prostrata*, *Phyllanthus amarus*, and others.

Notably, the absence of detectable levels of toxic metals (Pb, Cd, As, Cr) in *P. juliflora* is significant from a health safety perspective. These heavy metals, even in trace quantities, pose considerable health risks: Lead (Pb) affects neurological and cognitive functions, and Cadmium (Cd) is linked to kidney failure, lung disorders, and bone disease. Arsenic (As) may lead to circulatory collapse due to increased vascular permeability. Chromium (Cr) has bioaccumulative potential and can cause multiple organ damage and various cancers (Balali-Mood *et al.,* 2021).

The method validation results (CVs < 20%, $R^2 > 0.990$, acceptable recovery within 70 to 120%) confirm that the ICP-MS method employed is reliable and reproducible for multielement analysis. The measured uncertainties also fall within acceptable analytical limits. Overall, the findings underscore *P. juliflora*'s potential as a source of beneficial trace elements while posing minimal risk from heavy metal contamination.

5. Conclusion

The analytical method was validated for identifying 19 elements in *P. juliflora* based on the performance criteria of the instrument validation parameters. ICP-MS is a sensitive, accurate approach for identifying heavy metal content in *P. juliflora*. The data generated from the study gives an overview of the 19 elements, including heavy metals, found in *P. juliflora* leaves, bark, and pods. Mn, Cu, and Rb concentrations were detected at higher concentrations in *P. juliflora* leaves. All other remaining 15 elements, including heavy toxic metals such as Pb, As, Cd, and Cr were found to be below the LOQ level. The information makes it evident that *P. juliflora* leaves may be used in culinary and herbal preparations from an elemental perspective.

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Authors contributions

Nagaraj M Naik: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Writing-original draft, Writing-review and editing. Vasant Kumar: Methodology, Data curation, Formal analysis, Writing-original draft. Prabhuraj A: Conceptualization, Investigation, Supervision. Basavaraj S B: Data curation, Supervision. Ambrish G: Investigation, Methodology, Saraswati Mahato: Methodology, Data curation, Writing-original draft, Udaykumar Nidoni: Writing–review and editing, Supervision

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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