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Cation Profiles in Different Anatomical Parts of the Medicinal Plant Sambiloto (*Andrographis paniculata*) Determined by Ion Chromatography

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ABSTRACT. The cation composition of medicinal plants provides important information to characterize differences among their anatomical parts. This study determined six target cations, Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}, in the roots, stems, seeds, and leaves of Sambiloto (Andrographis paniculata) using ion chromatography (IC). Analysis was performed with a Metrohm Eco IC equipped with a Metrosep C4-150/4.0 column and a conductivity detector. The HNO_3 eluent concentration was optimized to 2.5 mM for separation at a flow rate of 0.8 mL/min and an injection volume of 10 µL. The method showed good analytical performance, with $R^2 > 0.998$, LODs of 0.024 – 0.284 mg/L, and RSDs of 1.89–3.04%. K+ was the predominant cation in all anatomical parts, ranging from 478.6 to 877.2 mg/kg. The highest Mg^{2+} concentration occurred in leaves (514.9 mg/kg), NH_4^+ in seeds (147.9 mg/kg), and Ca^{2+} in stems (149.3 mg/kg) and leaves (138.5 mg/kg). Na^+ was detected at moderate levels, while Li^+ was not detected. Total cation contents were highest in leaves (1,554.5 mg/kg), followed by stems (1,196.6 mg/kg), seeds (1,089.3 mg/kg), and roots (730.1 mg/kg). These results demonstrate the effectiveness of IC for characterizing cation profiles across Sambiloto anatomical parts.

INTRODUCTION

Andrographis paniculata, also known as Sambiloto, is a medicinal plant used in Indonesia and various other Asian countries, including India, Thailand, and China (Hossain *et al.*, 2014). This plant has long been used in traditional medicine to treat various diseases, including fever, diabetes, hypertension, liver disorders, infections, and related systemic immune diseases (Boro *et al.*, 2026). The pharmacological activity of Sambiloto is generally attributed to secondary metabolites, particularly andrographolide, flavonoids, diterpenoids, and other phenolic compounds (Avula *et al.*, 2025). Various studies have shown that these compounds possess anti-inflammatory, antioxidant, antibacterial, antiviral, antidiabetic, and immunomodulatory properties, making Sambiloto a promising medicinal plant for the development of phytopharmaceutical products (Zhang *et al.*, 2025; Boro *et al.*, 2026).

Besides the content metabolites, Sambiloto also contains various inorganic cations that play an important role in plant and human metabolism and health. These minerals are absorbed from the soil through the root system and distributed to various plant organs according to their physiological function. Among the cations present in plants, lithium, sodium, ammonium, potassium, magnesium, and calcium are important in various biological processes (Cao *et al.*, 2025). Potassium ions contribute to osmotic pressure and enzyme activation; magnesium ions are components of chlorophyll and are cofactors in various metabolic reactions, whereas calcium ions are involved in cell wall formation and signaling mechanisms. Sodium and ammonium ions also contribute to the balance of ionic

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and nitrogen metabolism in plants (Wathar and Verbruggen, 2026). For human health, these cations, known as electrolytes, play an essential role in maintaining fluid balance, transmitting nerve impulses, regulating muscle contractions, blood pressure, and energy metabolism, and participating in various enzymatic activities (Mahendra, 2023). Magnesium and calcium ions, for example, play important roles in healthy bones and neuromuscular function, while potassium and sodium ions help maintain electrolyte balance and cardiovascular function. Therefore, plant medicines containing essential cations are not only a potential source of bioactive compounds but also a natural source of these minerals, offering additional health benefits (Kumari *et al.*, 2025).

Until now, research on Sambiloto has been dominated by studies on secondary metabolites, especially andrographolide and phenolic compounds. In contrast, information on its cation profiles across different anatomical parts remains very limited. Therefore, Sambiloto was selected due to its widespread medicinal use and the need for a systematic comparison of cation profiles across its anatomical parts. Previous studies on medicinal plants have largely focused on total elemental or mineral contents, while comparative information on individual cationic species among different anatomical parts remains limited. In Sambiloto, comparative data on Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+} in roots, stems, seeds, and leaves remain limited. As the present study focuses on cation profiling, the relationship between cation levels and bioactive metabolites was not assessed and remains an important area for future research.

Determination of mineral contents in plant samples has commonly involved elemental techniques, including Atomic Absorption Spectroscopy (AAS) (Movsisyan *et al.*, 2026) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Kojo *et al.*, 2025). These techniques generally determine elemental contents following sample digestion. In contrast, IC enables the separation and simultaneous determination of several ionic species (Small, 1989; Amin *et al.*, 2008, 2023, 2024; Balcerzak and Janiszewska, 2015). This distinction is analytically important because IC provides information on ionic composition rather than merely serving as an alternative to total elemental analysis. Therefore, rather than replacing established elemental techniques, IC can provide complementary analytical information on water-extractable cationic species in plant extracts. In the present study, cold-water maceration was employed; therefore, the measured cation concentrations represent the water-extractable fraction under the applied extraction conditions rather than the total elemental content of the plant material.

Based on the above considerations, this study aims to simultaneously determine six target cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}) in the roots, stems, seeds, and leaves of Sambiloto using IC. These cations were selected to represent monovalent and divalent ionic species that can be determined simultaneously and to enable comparison of cation profiles among different anatomical parts of the plant.

RESEARCH METHODS

Chemicals and Standard Solutions

All chemicals used in this study were of analytical grade and obtained from Merck (Darmstadt, Germany). Lithium chloride (LiCl), sodium chloride (NaCl), ammonium nitrate (NH_4NO_3), potassium chloride (KCl), magnesium chloride ($MgCl_2$), and calcium chloride ($CaCl_2$) were used to prepare the corresponding Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+} stock standard solutions, respectively. Appropriate amounts of each salt were accurately weighed and dissolved in ultrapure water to prepare individual stock standard solutions containing 1000 mg/L of the respective target cation. Working mixed-cation standard solutions were subsequently prepared by appropriate dilution and mixing of the individual stock solutions with ultrapure water. Five concentration levels were prepared for calibration, ranging from 2 to 10 mg/L for Li^+ and from 4 to 20 mg/L for Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}, as presented in Table 1. Nitric acid (HNO_3, Merck, Darmstadt, Germany) was used to prepare the eluent for cation separation. The HNO_3 eluent solutions were prepared at the required concentrations by appropriate dilution with ultrapure water. HNO_3 eluent concentrations of 2.5, 3.0, and 3.5 mM were evaluated, and 2.5 mM HNO_3 was subsequently selected based on the cation separation performance.

Instrumentation and Chromatographic Conditions

Cation analysis was performed using a Metrohm Eco IC system equipped with a conductivity detector and a Metrosep C4-150/4.0 analytical column. The separation was performed using 2.5 mM HNO_3 as the eluent at a flow rate of 0.8 mL/min, with an injection volume of 10 µL. Under these chromatographic conditions, Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+} were separated and determined by conductivity detection.

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Plant Material and Sample Collection

Sambiloto (*Andrographis paniculata*) samples were collected from wild-growing plants in Tidore Islands City, North Maluku, Indonesia (Figure 1). The collected plants were separated into four anatomical parts, namely roots, stems, seeds, and leaves, and each part was subsequently prepared separately for cation analysis by ion chromatography (Figure 2).



Figure 1. Sambiloto plant collected from Tidore Island, Indonesia, was used as the sample material in this study.



(a) (b) (c) (d)

Figure 2. Separated anatomical parts of Sambiloto used for cation determination: (a) roots, (b) stems, (c) seeds, and (d) leaves. Each part was processed individually and analyzed to evaluate the distribution of essential cationic minerals.

Extraction and Sample Preparation

Each plant part was thoroughly washed with ultrapure water to remove soil, dust, and external ionic contaminants. Samples were then dried at room temperature and ground into a fine powder using a stainless-steel grinder. A total of 5.0 g of powder from each part of the plant was weighed and placed into a 100 mL tube. Next, 50 mL of ultrapure water was added to each tube, and the mixture was extracted by cold-water maceration at 25 ± 2 °C for 48 h. After the extraction process was finished, the mixture was centrifuged at 1000 rpm for 10 minutes to separate the residue from the liquid. The supernatant was obtained and filtered through a 0.22 µm membrane before IC analysis. The resulting extract from the fourth part of the anatomy of the plant Sambiloto is shown in Figure 3.

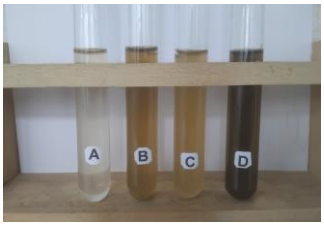


Figure 3. Cold-water extracts of different anatomical parts of Sambiloto after 48 hours of maceration at room temperature. From left to right: (A) root extract, (B) stem extract, (C) seed extract, and (D) leaf extract.

RESULTS AND DISCUSSION

Optimization of Eluent Concentration and Separation Performance of Cations

The separation of six cations, including lithium, sodium, ammonium, potassium, magnesium, and calcium ions, is affected by the eluent concentration. Therefore, the optimization uses three nitric acid concentrations: 2.5 mM, 3.0 mM, and 3.5 mM. The chromatogram of the standard solution mixture at various eluent concentrations is shown in Figure 4. In the 2.5 mM HNO_3 eluent (Figure 4a), all target ions were successfully separated, yielding sharp, well-resolved peaks and clear identification. Resolution between adjacent ions, especially sodium and ammonium ions, can still be maintained, preventing significant peak overlap. In addition, the baseline is relatively stable and returns to baseline, beginning quickly after elution of each ion, indicating a stable system with good chromatography.

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When the eluent concentration was increased to 3.0 mM HNO_3 (Figure 4b), the retention times of all ions decreased, thereby increasing the elution strength. However, the resolution between sodium and ammonium begins to decrease, so the two appear closer together. In this condition, baseline conductivity is also starting to improve, indicating an increased contribution of the eluent to the signal background. At a concentration of 3.5 mM HNO_3 (Figure 4c), the retention time decreased, but the separation quality decreased. The resolution between sodium and ammonium deteriorates, and several peaks broaden, potentially affecting the accuracy of quantitative analysis. In addition, reduced baseline stability was observed at 3.5 mM HNO_3, resulting in a longer time for the baseline to return to stability after elution. The results show that the improved concentration eluent consistently yields higher-quality and greater separation while reducing determination time.

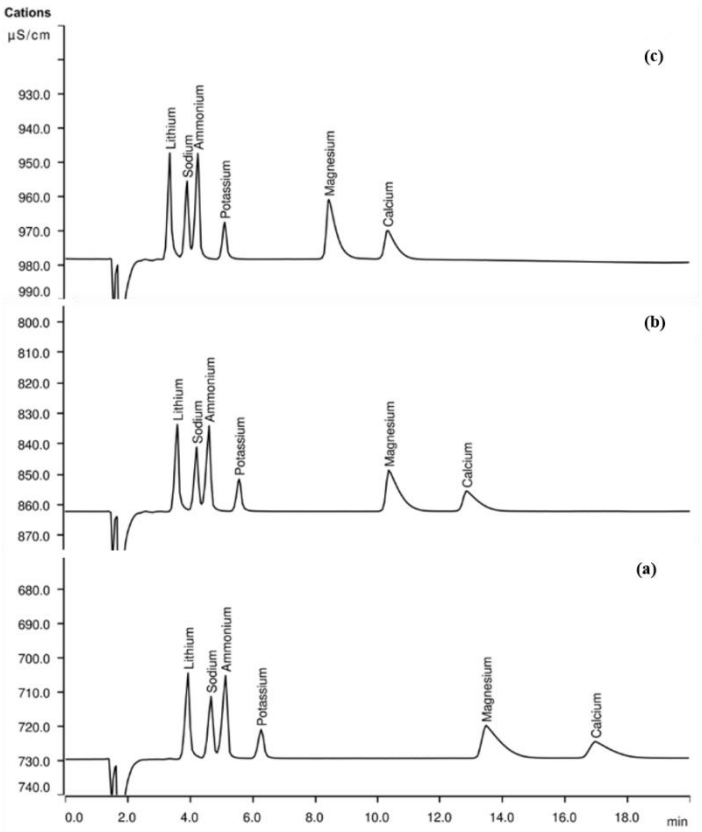


Figure 4. Ion chromatograms of standard cation mixture determined using IC under different eluent concentrations: (a) 2.5 mM HNO_3, (b) 3.0 mM HNO_3, and (c) 3.5 mM HNO_3. The x-axis represents retention time (min), and the y-axis represents conductivity (µS/cm). Cation concentrations (mg/L): lithium (4), sodium (8), ammonium (8), potassium (8), magnesium (8), and Calcium (8). Column separation: Metrosep C4-150/4.0. Eluent flow rate: 0.8 mL/min. Loop injection volume: 10-µL. Detector: conductivity detector.

Based on the chromatographic performance, increasing the HNO_3 concentration from 2.5 to 3.5 mM reduced cation retention times by increasing eluent strength and H^+ competition for ion-exchange sites. Although higher concentrations resulted in faster elution, 2.5 mM HNO_3 achieved better separation of the six cations and was therefore selected for further analysis. Under these optimum conditions, the separation of six cations was successfully achieved using a Metrosep C4-150/4.0 column and a conductivity detector. The retention times of each ion are about 3.5 minutes for lithium ion, 4.2 minutes for sodium ion, 5.0 minutes for ammonium ion, 6.0 minutes for potassium ion, 13.0 – 14.0 minutes for magnesium ion, and 16.0 – 17.0 minutes for calcium ion. Monovalent cations eluted at a relatively short time, shorter than divalent, because the interaction is more electrostatically weak with the stationary phase cation exchanger.

Moreover, the peak chromatography shows a relatively symmetrical form with adequate resolution. No indication of co-elution and disturbance from other components, so identification and quantification of each ion can be done in a way. In addition, the system chromatography shows good stability, with minimal signal drift during determination. This performance shows that the combination of the Metrosep C4-150/4.0 column and a 2.5

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mM HNO_3 eluent is well-suited for determining cations in anatomical parts of the Sambiloto plant sample. The initial negative response observed at approximately 1 – 2 min was attributed to the system/injection peak rather than to any of the target cations. This response may arise from the conductivity difference between the injected aqueous solution and the HNO_3 eluent and was therefore excluded from cation identification and quantification.

Method Validation

Linearity

The linearity method was evaluated using five concentration levels for each cation (Table 2). Solution lithium standards were prepared over a concentration range of 2 – 10 mg/L, whereas solution sodium, ammonium, potassium, magnesium, and calcium ion standards were prepared over a concentration range of 4 – 20 mg/L. A total of 10 µL of each standard solution was injected three times to build a calibration curve.

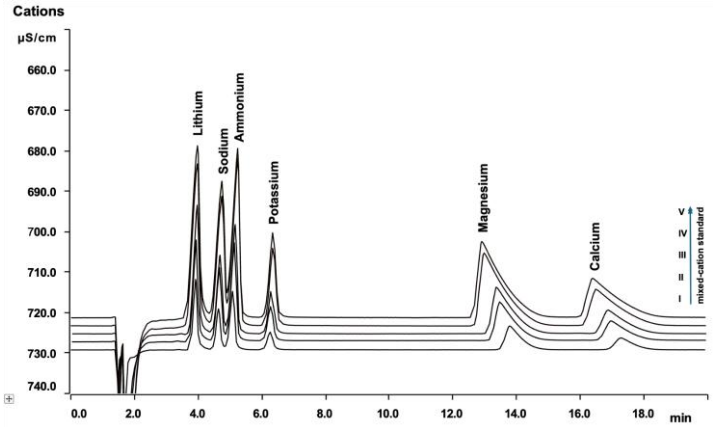


Figure 5. Ion chromatograms of mixed cation standard solutions (I – V) containing Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+} at the concentrations listed in Table 1. The x-axis represents retention time (min), and the y-axis represents conductivity (µS/cm). Chromatographic conditions were the same as those used in Figure 4a.

A calibration curve was obtained by plotting the peak area against the concentration of each ion. As shown in Table 1, all cations exhibit an excellent linear relationship between concentration and detector response over the tested range. The coefficient of determination (r^2) obtained is well above 0.998, indicating that this method exhibits excellent linearity for the quantitative determination of lithium, sodium, ammonium, potassium, magnesium, and calcium ions. These results indicate that the change-response detector directly compares changes in analyte concentration, making the method widely used for determining cationic minerals in plants such as Sambiloto.

Table 1. Concentrations of the mixed-cation standard solutions used for calibration.

Cations	I	II	III	IV	V
Li^+	2	4	6	8	10
Na^+	4	9	12	18	20
NH_4^+	4	9	12	18	20
K^+	4	9	12	18	20
Mg^{2+}	4	9	12	18	20
Ca^{2+}	4	9	12	18	20

Note: I–V represent five concentration levels of the mixed cation standard solutions used for calibration. Concentrations are expressed in mg/L.

Limit of Detection (LOD)

The sensitivity of the method was evaluated by determining the limit of detection (LOD) for each target cation, shown in Table S1. The LOD values ranged from 0.024 to 0.284 mg/L. The lowest LOD was obtained for Li^+ (0.024 mg/L), while the highest was obtained for Ca^{2+} (0.284 mg/L), as summarized in Table 2. The LOD values reflect varying sensitivities among the six cations. Higher LODs for Mg^{2+} and Ca^{2+} likely result from their broader, less symmetrical chromatographic peaks, which produce lower peak heights and reduced analytical sensitivity compared to monovalent cations. However, the method still offers sufficient sensitivity for analyzing the target cations in Sambiloto samples.

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Table 2. Data on the detection limit (LOD), relative standard deviation (RSD) of peak height, and retention time (t_R) under the chromatographic standard sample in Figure 4a.

Cations	LOD (mg/L)	RSD (%) of peak height (n = 5)	Retention time, t_R (min)
Li^+	0.024	1.89	3.94
Na^+	0.074	1.93	4.67
NH_4^+	0.056	2.35	5.13
K^+	0.161	2.65	6.27
Mg^{2+}	0.141	2.71	13.46
Ca^{2+}	0.284	3.04	16.96

Precision and Reproducibility of Retention Time

Precision was evaluated by calculation deviation standard relative (RSD) based on the peak height of 5 injections repeated (n = 5), shown in Table S2. The RSD values obtained range from 1.89% to 3.04% (Table 2). All values are still within the acceptable limits. Chromatographic determination shows that the method has good repeatability and adequate stability in the system during analysis.

Slight RSD value is higher in calcium ions, probably related to the fact that it has a greater retention time compared to other ions. Longer retention times can increase the opportunity for occurrence-variation signal detection and fluctuations in the condition system during the separation process. Thus, the RSD value obtained still indicates a level of precision for objective quantification. Reproducibility of retention time also shows satisfactory results. Retention times for all ions range from 3.94 minutes for lithium to 16.96 minutes for calcium, with very small variations between injections. Consistent retention time indicates that the chromatographic system operates stably during measurement. In addition to good separation and profile-consistent retention, the method is applied to matrix-complex plants, such as Sambiloto.

Evaluation of Overall Method Performance

Based on the results for linearity, sensitivity, precision, and reproducibility, the time retention method developed for IC shows excellent analytical performance in determining six essential cationic minerals. A high R^2 value ($R^2 > 0.998$), a low LOD, and RSD values below 5% indicate that the method meets the condition-based validation criteria for quantitative analysis. Therefore, under the analytical conditions evaluated in this study, the method was suitable for determining the cation profiles in different anatomical parts of Sambiloto.

Application of Methods to Various Anatomical Parts of the Sambiloto Plant

The IC method has been validated and further applied to determine the cationic mineral content in various parts of the Sambiloto plant's anatomy. Chromatogram extract roots, stems, seeds, and leaves, shown in Figure 6. The results of the analysis show that the five target cations, lithium, sodium, ammonium, potassium, magnesium, and calcium ions, can be separated and determined with a good overall sample.

On the root chromatogram (Figure 6a), the magnesium ion peak shows a shorter retention time than the others. This phenomenon is allegedly due to the high concentration of magnesium ions in the samples, which causes column overloading and the formation of a non-symmetrical peak with a long tail. The peak was distorted, with the maximum detected earlier, resulting in a shorter observed retention time. A similar tendency is observed in the chromatogram of the standard mixture (Figure 5), where an increase in the concentration of divalent cations results in higher peak heights and earlier peak maxima.

Based on the results, sodium ions were detected in roots (51.6 mg/kg), stems (50.2 mg/kg), and leaves (46.4 mg/kg), while their content in seeds was much lower (7.4 mg/kg). The distribution shows that sodium ions are more concentrated in the network vegetative tissue than in the reproductive organs. Sodium ions play a role in osmosis, ion balance, and the maintenance of cell turgor. Distribution and comparison of sodium content in each part of the anatomy of Sambiloto are presented in Figure 7a. Ammonium ion distribution is uneven, with the highest concentration in seeds (147.9 mg/kg), followed by leaves (13.4 mg/kg), stems (10.8 mg/kg), and roots (4.7 mg/kg). The high ammonium ion content in seeds indicates sufficient nitrogen storage to support the germination and growth of young plants. Distribution and comparison of the content of ammonium in each part of the anatomy of Sambiloto are shown in Figure 7b. Potassium ion is cation-dominant throughout the plant, with concentrations ranging from 478.6 mg/kg in roots to 877.2 mg/kg in the stem. Potassium ions play important roles in enzyme activation, stomatal regulation, osmoregulation, and the translocation of photosynthetic products. The high potassium content in the stem suggests a role in nutrient transport and storage. Distribution and comparison of

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potassium ion content in each part of the anatomy of Sambiloto are shown in Figure 7c. Magnesium ion shows the highest in leaves (514.9 mg/kg), followed by seeds (113.2 mg/kg), stems (109.1 mg/kg), and roots (79.8 mg/kg). The high magnesium ion content in leaves is related to its function as the central ion in the chlorophyll molecule and as a cofactor for various enzymes involved in photosynthesis and energy transfer. Distribution and comparison of magnesium ion content in each part of the anatomy of Sambiloto are presented in Figure 7d.

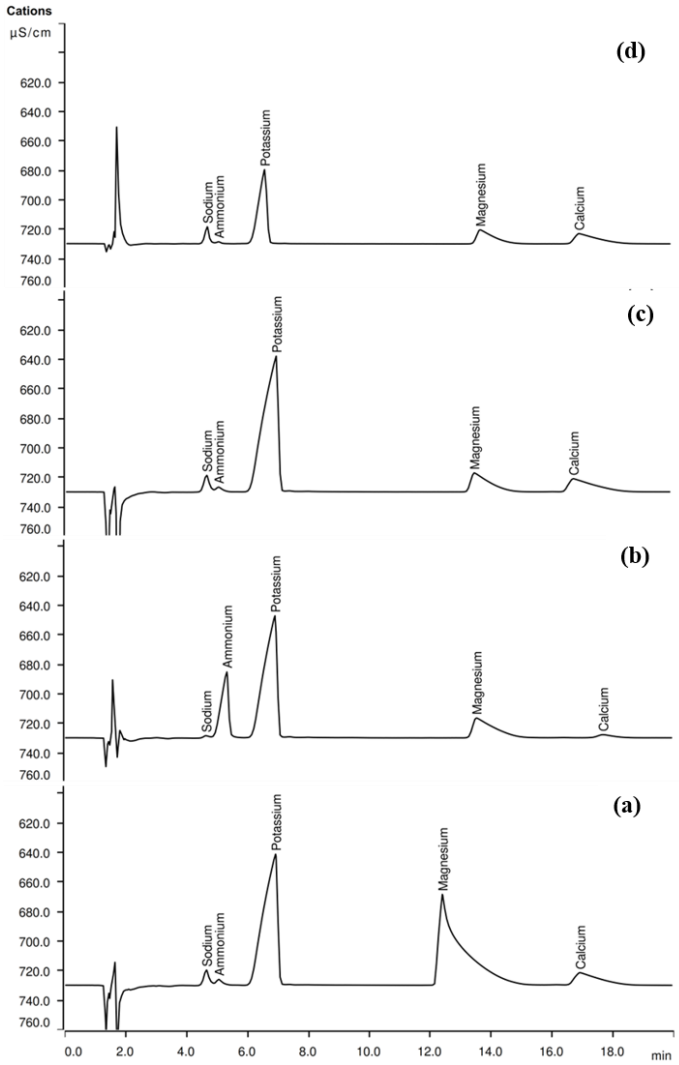


Figure 6. Ion chromatograms representing the concentrations of essential cationic minerals (lithium, sodium, ammonium, potassium, magnesium, and calcium ions) detected in four anatomical parts of Sambiloto: (a) root, (b) stem, (c) seed, and (d) leaf. Other identical ion chromatography conditions as in Figure 4a.

Calcium ions were detected throughout the plant, with the highest concentrations in stems (149.3 mg/kg) and leaves (138.5 mg/kg), followed by roots (115.4 mg/kg), and the lowest in seeds (35.2 mg/kg). Calcium ions play an important role in stabilizing the cell wall, maintaining the integrity of cellular membranes, and regulating mobile signaling processes. Because of its relatively low mobility in the phloem, elements tend to accumulate in active transpiration networks, such as stems and leaves. Distribution and comparison of calcium ion content in every part of the anatomy of Sambiloto is shown in Figure 7e.

Lithium ions were not detected throughout the plant under the conditions analyzed. These results show that the lithium ion content in Sambiloto is very low or below the method's LOD. In addition, lithium ions are essential to plants, so their absorption from the environment is generally very limited. Slight shifts in the retention times of some cations were observed among the Sambiloto extracts, which may be attributed to matrix effects arising from differences in the composition and concentration of coexisting ionic components. Peak identification was therefore based on comparison with the retention times of the corresponding cation standards analyzed under the same chromatographic conditions.

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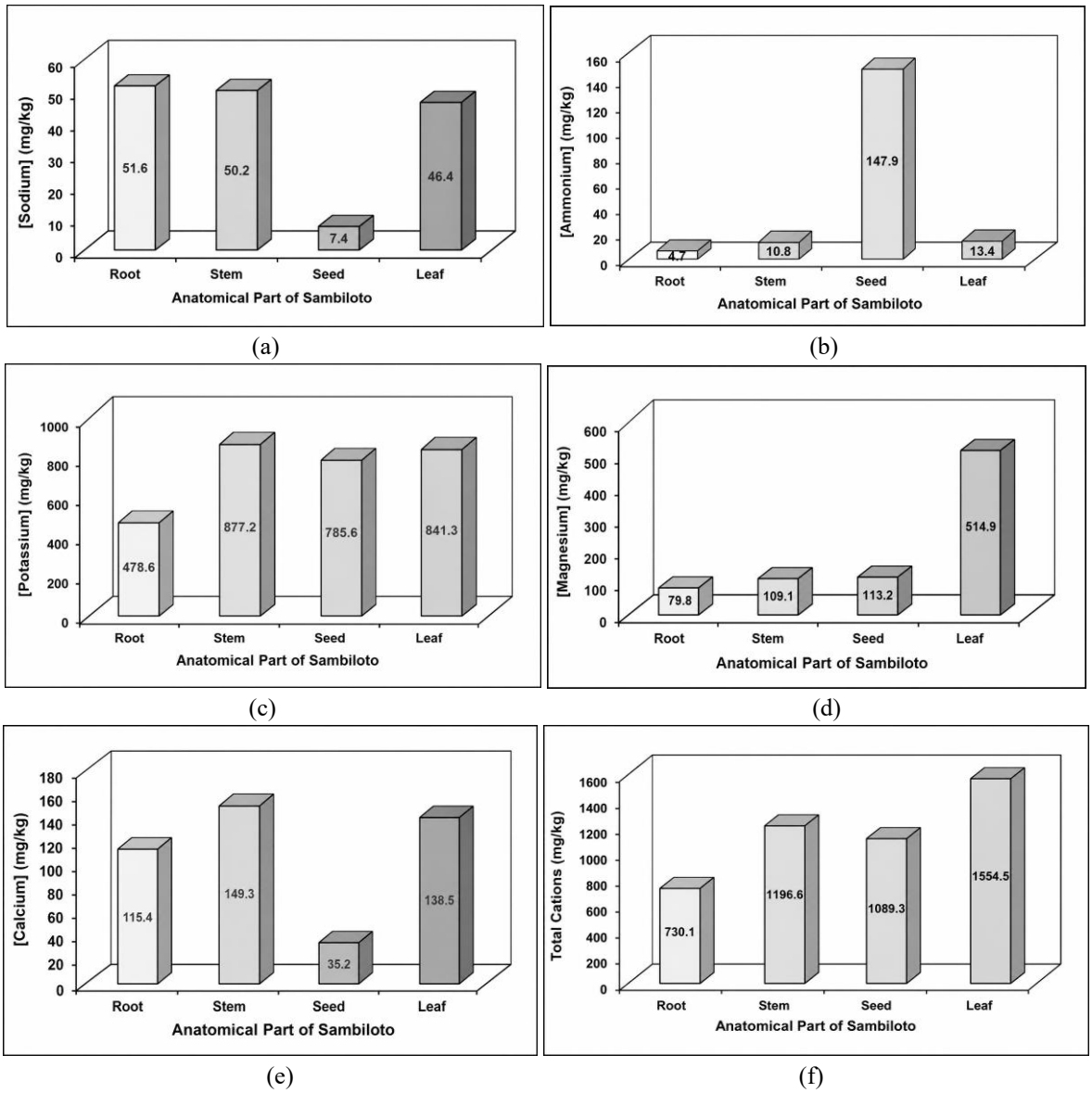


Figure 7. Cation contents determined in different anatomical parts of Sambiloto (root, stem, seed, and leaf): (a) sodium, (b) ammonium, (c) potassium, (d) magnesium, (e) calcium, and (f) total cations.

Cation Content in Different Anatomical Parts of Sambiloto

Figure 7f shows the total water-extractable cation content determined in different anatomical parts of Sambiloto. The total water-extractable cation content varied among plant parts, with the highest in the leaves (1554.5 mg/kg), followed by the stems (1196.6 mg/kg), seeds (1089.3 mg/kg), and roots (730.1 mg/kg). The higher value in the leaves was mainly associated with their substantially higher Mg^{2+} content (514.9 mg/kg) and high K^{+} content (841.3 mg/kg). Differences in cation contents among plant organs may be related to their distinct physiological functions and ion requirements. K^{+} plays important roles in plant osmotic regulation, water balance, and the activation of numerous enzymes, whereas Mg^{2+} is the central atom of the chlorophyll molecule and is essential for photosynthetic processes (Johnson *et al.*, 2022; Ahmed *et al.*, 2023). These physiological functions may partly explain the relatively high levels of K^{+} and Mg^{2+} observed in Sambiloto leaves. However, cation accumulation in individual plant organs can also be influenced by ion availability, uptake, transport, and environmental conditions. Since these factors were not specifically investigated in the present study, the observed differences should be interpreted as variations in water-extractable cation profiles rather than direct evidence of specific ion-accumulation mechanisms (de Bang *et al.*, 2021).

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CONCLUSION

An IC method was successfully applied to determine six target cations, including Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}, in water extracts of the roots, stems, seeds, and leaves of Sambiloto. The developed method demonstrated satisfactory analytical performance, with coefficients of determination (R^2) above 0.998, LOD values ranging from 0.024 to 0.284 mg/L at S/N = 3, and RSD values ranging from 1.89 to 3.04% for five replicate injections (n = 5). The analyzed plant parts showed distinct profiles of water-extractable cations. K^+ was the predominant cation in all anatomical parts, with the highest concentration observed in the stems (877.2 mg/kg), while Mg^{2+} showed its highest concentration in the leaves (514.9 mg/kg). NH_4^+ was highest in the seeds (147.9 mg/kg), whereas Ca^{2+} was relatively high in the stems (149.3 mg/kg) and leaves (138.5 mg/kg). Na^+ was detected in all anatomical parts, whereas Li^+ was not detected under the applied analytical conditions. The total water-extractable cation content was highest in the leaves (1,554.5 mg/kg), followed by the stems (1,196.6 mg/kg), seeds (1,089.3 mg/kg), and roots (730.1 mg/kg). These findings provide analytical information on variations in water-extractable cation profiles among the anatomical parts of Sambiloto under the applied extraction conditions. Further studies are recommended to cross-validate the IC method using an independent analytical technique, such as AAS or ICP-MS, and to evaluate the relationship between water-extractable cation concentrations and total elemental contents in Sambiloto.

SUPPLEMENTARY FILE

Tables S1 and S2 are available in the Supplementary Information and accessible at https://jurnal.uns.ac.id/alchemy/article/view/120387/supp.info.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

MA: Conceptualization, Methodology, Formal Analysis, Writing – Original Draft, Writing – Review and Editing and approval of the final manuscript; ES and LKM: Methodology, Validation, Formal Analysis, Writing – Review and Editing and approval of the final manuscript; AS: Formal Analysis, Writing – Review and Editing; MM and ARI: Investigation, Sample Preparation; KAR: Data Curation, Visualization, Writing – Review and Editing.

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DECLARATION OF GENERATIVE AI

The authors used ChatGPT (OpenAI) for English-language editing, grammar correction, and text clarity during manuscript preparation and revision. The tool was not used to generate research data or experimental results. All AI-assisted content was reviewed and verified by the authors, who take full responsibility for the final manuscript.

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