Bioactive potential of *Salvia officinalis* leaves and stems mix from commercial and fresh samples

Mariana Ferreira dos Santos [1,2], *Alex de Aguiar Novo* [2,3], *Claudete Norie Kunigami* [2,4], *Eliane Przytyk Jung* [2,5] & *Leilson de Oliveira Ribeiro* [2,6,7]

Key words:
- antioxidants
- infusion
- phenolic compounds
- Sage

Abstract

Salvia officinalis is a plant widely consumed due to its biological properties, among which its antioxidant activity stands out. In this study, infusions prepared from a mixture of leaves and stems of the commercial and botanically identified plant were analyzed to evaluate their total phenolic compounds (TPC) and antioxidant capacity. The results, in dry basis (d.b.), demonstrated that the infusions present significant concentrations of phenolic compounds and high antioxidant capacity, with the highest values of TPC and antioxidant capacity found respectively for the commercial sample: 2164 mg GAE/100 g, 149 µmol Trolox/g, 141 µmol Trolox/g, and 445 µmol Fe^{2+}/g ($ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP, respectively). For the botanically identified samples, the highest values found were 1884 mg GAE/100 g for TPC, and 48 µmol Trolox/g, 66 µmol Trolox/g, and 277 µmol Fe^{2+}/g ($ABTS^{\bullet+}$, $DPPH^{\bullet}$ and FRAP, respectively). Infusions of *S. officinalis* prepared with leaves and stems are little explored in the literature, proving the findings of this study to be relevant, as it faithfully reflects how the plant is used in the homemade preparation of teas/infusions. Thus, this study contributes to a better understanding of the bioactive potential of *S. officinalis*, reinforcing its potential application as a natural source of antioxidants.

Palavras-chave:
- antioxidantes
- infusão
- compostos fenólicos
- Sálvia

Resumo

Salvia officinalis é uma planta amplamente consumida devido às suas propriedades biológicas, dentre as quais se destaca a atividade antioxidante. Neste estudo, infusões preparadas a partir de uma mistura de folhas e caules da planta comercial e botanicamente identificada foram analisadas para avaliar o teor de compostos fenólicos e capacidade antioxidante. Os resultados, em base seca, demonstraram que as infusões apresentam concentrações significativas de compostos fenólicos e alta capacidade antioxidante, com os maiores valores encontrados para a amostra comercial de 2164 mg GAE/100 g para TPC e 149 µmol Trolox/g, 141 µmol Trolox/g e 445 µmol Fe^{2+}/g ($ABTS^{\bullet+}$, $DPPH^{\bullet}$ e FRAP, respectivamente). Para as amostras identificadas botanicamente, os maiores valores encontrados foram 1884 mg GAE/100 g para TPC e 48 µmol Trolox/g, 66 µmol Trolox/g e 277 µmol Fe^{2+}/g ($ABTS^{\bullet+}$, $DPPH^{\bullet}$ e FRAP, respectivamente). Infusões de *S. officinalis* preparadas com folhas e caules são pouco exploradas na literatura, ressaltando-se aqui a importância dos achados deste estudo que reflete com fidelidade à forma como a planta é utilizada no preparo caseiro de chás e infusões. Assim, este estudo contribui para uma melhor compreensão do potencial bioativo de *S. officinalis*, reforçando seu potencial de aplicação como fonte natural de antioxidantes.

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[1] Federal Institute of Education, Nilópolis, RJ, Brazil. ORCID: 0009-0001-8836-2422
[2] National Institute of Technology, Rio de Janeiro, RJ, Brazil.
[3] ORCID: 0009-0000-5634-7183
[4] ORCID: 0009-0002-8612-8672
[5] ORCID: 0000-0002-2376-229X
[6] ORCID: 0000-0003-3252-4987
[7] Author for correspondence: leilson.oliveira@int.gov.br

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Introduction

Herbal infusions, known as teas, have been widely consumed throughout history. Sage (*Salvia officinalis* L.) is a perennial and aromatic plant belonging to the Lamiaceae family, recognized for its medicinal and culinary properties (Khan & Abourashed 2010; Longe 2005). Its name derives from the Latin *salvare*, meaning 'to heal', reflecting its long history of medicinal use by different cultures. The plant is also known as a sacred herb, European or Greek tea (Waltch *et al*. 2011).

Native from the Mediterranean region and mainly acclimated in the southern region of Brazil (Povh & Ono 2008), this species is rich in secondary metabolites, such as flavonoids, terpenoids, and phenolic acids, which confer a series of biological activities (Yaman 2020). Among these compounds, phenolics are particularly relevant due to their antioxidant action, which can protect against oxidative damage, delay cellular aging and prevent diseases associated with oxidative stress, such as neurodegenerative and cardiovascular diseases (Ness & Powles 1997).

Scientific evidence indicates that sage infusion has high antioxidant potential, due to containing compounds such as rosmarinic acid, carnosic acid, carnosol, luteolin, and apigenin. These compounds are essential in neutralizing free radicals and reducing oxidative stress in the human organism (Zimmermann *et al*. 2011).

Studies conducted by Walch *et al*. (2011) and Hamidpour *et al*. (2014) suggest that regular consumption of infusions of the plant can contribute to protection against metabolic diseases, while also improving cognitive functions and reducing inflammatory processes. Other researches, such as that carried out by Bommer *et al*. (2009), also highlights the effectiveness of *S. officinalis* in treating hot flashes during menopause. A double-blind, randomized, placebo-controlled study by Akhondzadeh *et al*. (2003) demonstrated that sage could improve memory and cognitive function in individuals with mild to moderate impairment from Alzheimer's disease.

Despite the biological effects of *S. officinalis*, the composition of infusions from commercial samples can vary significantly, depending on factors such as the part of the plant used (leaves or stems), industrial processing, and harvest seasonality (Francik *et al* 2020).

Several studies have shown that different parts of medicinal plants, such as leaves and stems, have distinct profiles of bioactive compounds, directly influencing their biological activity. In a study with Lemon Balm (*Melissa officinalis* L.), leaf extracts showed a significantly higher content of total phenolic compounds (32.76 mg GAE/g) compared to stems (8.4 mg GAE/g) as well as antioxidant activity (Moacã *et al*. 2018). Similarly, in the evaluation of Yerba Mate (*Ilex paraguariensis* A.St.-Hil.) leaf extracts showed the highest antioxidant activity, correlated with high levels of phenolic compounds compared to stems. On the other hand, stem extracts showed higher anti-inflammatory potential (Souza *et al*. 2015).

In the study of infusions prepared from different aerial parts of *Portulaca oleracea* L. the results demonstrated that stem infusions had significantly higher total phenolic content and antioxidant capacity compared to leaf and flower infusions. This reinforces the importance of considering different parts of the plant, such as stems, when evaluating the bioactive potential of infusions. (Silva & Carvalho 2014).

Thus, the presence of stems, often found in commercial products, can affect the amount of phenolic compounds available in the infusion, directly affecting its antioxidant capacity, presenting different results from those obtained using only the leaves to prepare of this product. Therefore, in order to guarantee the benefits attributed to the plant and identify possible effects, it is important to evaluate the quality of the product that reaches the consumer's home.

This study aims to evaluate the antioxidant capacity (AC) and the total phenolic compounds (TPC) content of *S. officinalis* infusions prepared from different commercial brands, comparing them with samples of the botanically identified plant collected in various seasons of the year.

Materials and Methods

Samples

Fresh plants of *S. officinalis*, botanically identified and cultivated in the metropolitan region of the state of Rio de Janeiro, Brazil (-22.514956, -43.187215), were harvested during four different climatic seasons (spring, summer, autumn, and winter) in 2023/2024, totaling four botanical samples. Leaves and stems were manually separated, dried in an oven at 45 °C for 24 hours, and subsequently ground using a domestic blender. All samples presented ~10% of moisture.

Ten commercial samples of *S. officinalis* were obtained from local stores in the city of Rio de Janeiro, comprising products from five different commercial brands and one sample not branded (bulk). Among the commercial samples, one was represented by four batches, another by two batches, and the remaining four by a single batch each. Leaves and stems were manually separated and the material was crushed using a domestic mixer.

To standardize the samples, all commercial materials were adjusted to a composition of 65% crushed leaves and 35% crushed stems, reflecting the average proportion found in the commercial samples. After mixing these fractions, the samples were used to prepare the infusions.

Infusions

The infusions were made at 5% (g/mL), adding 25 mL of boiling ultrapure water to 1.25 g of each sample. After 20 minutes, extracts were filtered and their contents were transferred to a 25 mL volumetric flask and adjusted with ultrapure water. The infusions were stored frozen at -18 ºC until the analyses.

Total phenolic compounds (TPC)

This analysis was performed using Folin-Ciocalteu reagent, according to the method described by Singleton & Rossi (1965). For that, 250 μL of each extract were reacted with 1,250 μL of 10% Folin-Ciocalteu (v/v) and 1,000 μL of 7.5% Na_2CO_3 (w/v). Subsequently, the mixtures were heated to 50 °C for 15 minutes, being cooled in an ice bath to read the absorbance at 760 nm with a spectrophotometer UV-Vis (SP-220, Biospectro, Curitiba, Brazil). The content of TPC in the extracts was expressed as mg gallic acid equivalents per 100 g of sample in dry basis (mg GAE/100 g, d.b.). Also, the results were expressed as mg GAE/200 mL of infusion.

DPPH• assay

The DPPH• method was performed according to the methodology proposed by Hidalgo *et al.* (2010). For the reactions, 100 μL of each extract was added to 2,900 μL of DPPH• solution (6×10^{-5} M) in methanol and diluted to obtain an absorbance of 0.700 at 517 nm) and allowed to react for 30 minutes in the dark, at room temperature. The absorbances were measured at 517 nm with a spectrophotometer UV-Vis (SP-220, Biospectro, Curitiba, Brazil). Results were obtained by preparing a Trolox standard curve. They were expressed as µmol Trolox/g d.b. Also, the results were expressed as µmol Trolox/200 mL of infusion and, to compare with the literature, the calculation of the inhibition percentage was performed in according to the equation:

$$\% \text{ inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control} \times 100]$$

ABTS•+ assay

The antioxidant capacity of the extracts was determined by the ABTS•+ assay according to the methodology proposed by Gião *et al.* (2007). There was a reaction of 30 μL of the extract with 3 mL of the ABTS•+ radical solution in the dark at room temperature for 6 minutes. The reaction was carried out in the absence of light to avoid photodegradation of the ABTS•+ radical. The results were obtained from the preparation of a Trolox standard curve, and the absorbances were read in a spectrophotometer at 734 nm with a spectrophotometer UV-Vis (SP-220, Biospectro, Curitiba, Brazil). The results were expressed as µmol Trolox/g d.b. Also, the results were expressed as µmol Trolox/200 mL of infusion.

FRAP assay

The analysis was performed according to Benzie & Strain (1996) with slight modifications. The FRAP solution was made by mixing 25 mL of 300 mM acetate buffer (pH 3.6) with 2.5 mL of 10 mM TPTZ solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3 \bullet 6H_2O$. 100 μL of each extract were reacted with 3 mL of FRAP at 37 °C for 30 min. After this period, the absorbance was measured at 593 nm with a spectrophotometer UV-Vis (SP-220, Biospectro, Curitiba, Brazil). The results were obtained by preparing a standard curve of $FeSO_4.7H_2O$, and expressed as µmol Fe^{2+}/g d.b. Also, the results were expressed as µmol Fe^{2+}/200 mL of infusion.

Statistical analyses

The results were presented as a mean ± standard deviation. The infusions were prepared in duplicate and the analyses were performed in triplicate. Statistical analysis was performed using analysis of variance (ANOVA) followed by Tukey's test at a 95% confidence level, utilizing Statistica® version 13.0 software.

Results and Discussion

According to Godos and Grosso (2021), foods rich in antioxidants play a key role in disease prevention. Phenolic acids and flavonoids, for example, are the groups of compounds with the most antioxidant activity (Francik *et al.* 2020). These compounds participate in defensive processes during infection, excessive exposure to sunlight and plant injuries (Salunke & Koche 2023); therefore, these compounds are synthesized by plant in stress conditions (Munteanu & Apetrei 2021). This, associated with other factors, can cause heterogeneity in the bioactive composition and, consequently, in the antioxidant capacity of infusions prepared with medicinal plants.

Table 1 shows the results, in dry basis, obtained for TPC and antioxidant capacity by the DPPH•, ABTS•+ and FRAP methods for samples evaluated in the current study.

For TPC, the values ranged from 937 to 2164 mg GAE/100 g in the commercial samples (CS), while for the botanically identified samples (BS) the range was from 473 to 1,884 mg GAE/100 g.

Table 1 – Total phenolic content (TPC), and antioxidant capacity of infusions of commercial and botanically identified samples of *S. officinalis*.

Samples	Assays			
	TPC [1]	DPPH• [2]	ABTS•+ [2]	FRAP [3]
1 (CS1/1)	1023 ± 18[g]	72 ± 1[g]	70 ± 4[f]	232 ± 10[f]
2 (CS1/2)	2164 ± 16[a]	149 ± 4[a]	121 ± 4[b]	445 ± 7[a]
3 (CS1/3)	1633 ± 44[c]	108 ± 1[d,e]	101 ± 4[c]	322 ± 9[d]
4 (CS1/4)	1106 ± 26[f]	65 ± 2[h]	74 ± 4[e,f]	74 ± 3[h]
5 (CS2/1)	1626 ± 35[c]	107 ± 1[e]	115 ± 3[b]	319 ± 16[d]
6 (CS3/1)	2019 ± 36[b]	136 ± 3[b]	141 ± 15[a]	427 ± 4[b]
7 (CS3/2)	1316 ± 81[d]	115 ± 3[c]	102 ± 6[c]	392 ± 8[c]
8 (CS4/1)	937 ± 22[h]	63 ± 3[h]	65 ± 2[f]	217 ± 33[f]
9 (CS5/1)	1088 ± 10[f,g]	84 ± 2[f]	80 ± 3[d,e]	271 ± 10[e]
10 (CS6/1)	2142 ± 40[a]	112 ± 3[c,d]	87 ± 6[d]	422 ± 21[b]
11 (Spring)	888 ± 37[h]	48 ± 2[i]	66 ± 2[h]	217 ± 3[f]
12 (Summer)	473 ± 3[i]	13 ± 0[j]	26 ± 1[i]	54 ± 1[h]
13 (Autumn)	1884 ± 18[e]	47 ± 3[i]	54 ± 3[g]	176 ± 3[g]
14 (Winter)	524 ± 9[i]	14 ± 1[j]	19 ± 2[i]	68 ± 2[h]

Legend: different lowercase letters in the same column indicate that the results are statistically different ($p < 0.05$). 1 = results expressed in mg GAE/100 g; 2 = results expressed in μmol Trolox/g; 3 = results expressed in μmol Fe^{2+}/g. Sample 11 to 14: Botanically identified samples (BS); Commercial samples (CS); Commercial sample brand 1/batch 1 (CS1/1). Results expressed on a dry basis. Source: Prepared by the author, 2025.

Samples 2 and 10 presented the highest TPC values, indicating a high content of phenolic compounds.

Regarding the antioxidant capacity, it was observed that all samples showed potential against the tested radicals. The variation for the DPPH• method was from 63 to 149 μmol Trolox/g for CS, and 13 to 48 μmol Trolox/g for BS. For $ABTS^{\bullet+}$ the variation was from 65 to 141 μmol Trolox/g for CS, and 19 to 66 μmol Trolox/g for BS. Finally, for FRAP the values varied from 74 to 445 μmol Trolox/g for CS, and 54 to 217 μmol Trolox/g for BS.

The antioxidant capacity evaluated by the DPPH• and FRAP methods indicated that sample 2 stood out in terms of its antioxidant potential, which was consistent with its higher TPC value. By the $ABTS^{\bullet+}$ analysis, sample 6 presented the highest value among all samples. In contrast, samples 12 and 14, corresponding to the botanical samples collected in summer and winter, respectively, presented the lowest values in all tests performed (DPPH•, $ABTS^{\bullet+}$ and FRAP).

The CS showed variations both between different brands and between batches of the same brand ($p < 0.05$). These differences may be associated with factors such as the origin of the raw material, cultivation conditions, processing, and storage. In the study conducted by Amoo *et al.* (2012), it was observed that medicinal plants stored for 12 to 16 years in the dark at room temperature were able to retain their biological activity. The authors reported significantly higher TPC values in stored materials compared to fresh ones for species such as *Artemisia afra* Jacq. *ex* Willd., *Clausena anisata* (Willd.) Hook.f. *ex* Benth., *Cussonia spicata* Thunb., *Leonotis intermedia* Lindl., and *Spirostachys africana* Sond.

Ordoñez *et al.* (2020) also reported that the variation in antioxidant and polyphenol levels in commercial and botanical infusions can be influenced by several factors, such as the preparation method (temperature and time); the sample preparation method (*e.g.*, drying); the plant growth conditions; the analytical technique employed; the extraction method used; and the use of different parts of the plant, such as roots, leaves, stems, bark and flowers. This was corroborated by Pérez-Burillo *et al.* (2018), Saifullah *et al.* (2019) and Gallia *et al.* (2024).

For the BS, the highest values of TPC and antioxidant capacity were observed in spring and autumn. The differences can be explained by the different environmental conditions related to

each period of the year, such as sunlight, rainfall, temperature and biotic factors (Generalić *et al*. 2012). According to Gololo *et al*. (2016) the medicinal plants may have high accumulation of different phytochemicals during different seasons, which is in alignment with the results of the current study. These authors analyzed leaves of three medicinal plants (*Barleria dinteri* Oberm., *Grewia flava* DC. *and Jatropha lagarinthoides* Sond.) and noticed that alkaloids and tannins were in high quantities during the colder seasons (autumn and winter) in all plants, while flavonoids were in high amounts during the warmer seasons (spring and summer).

The analyses also revealed significant differences between the two groups of samples, which may be associated with some adulteration of the commercial samples and the differences among their harvest time, processing, and storage. These factors may affect the quality/quantity of the bioactive compounds in the infusions (Srirama *et al.* 2017; Lisboa *et al*. 2022; Gobbo-Neto & Lopes 2007). A study performed by Kumar *et al.* (2018) reported that, among 203 samples analyzed from 30 species of medicinal plants, approximately 12% presented adulterations, with substitution rates ranging from 20% to 100%, which demonstrates a lack of basic quality and safety control in the marketing of these products.

In general, the values of DPPH$^{\bullet}$, ABTS$^{\bullet+}$, and FRAP followed a similar trend to the TPC, suggesting a relationship between the phenolic content and the antioxidant activity as stated by Gallia *et al*. (2024) who highlight phenolic compounds as the main phytochemical compounds with antioxidant capacity in *Monttea aphylla* (Miers) Hauman, *Larrea cuneifolia* Cav., *Larrea divaricata* Cav., *Grindelia chiloensis* (Cornel.) Cabrera, *Pteromonnina dictyocarpa* (Griseb.) B.Eriksen and *Mandevilla laxa* (Ruiz & Pav.) Woodson.

According to Francik *et al*. (2020), infusions of fresh *S. officinalis* leaves showed values of 890 to 1,160 mg GAE/100 g for TPC. The results obtained in the current study for BS are close to the range reported by the authors, except for sample 13, which refer to autumn (1,884 mg GAE/100 g). For DPPH$^{\bullet}$, inhibition percentages of 36% to 42.2% were reported, which are also close to the range found (28%–33%) for BS diluted between 2–10x in the current study.

In a study on *S. officinalis* extracts, the TPC ranged from 2083 to 11931 mg GAE/100 g, when the sample was extracted using different solvents and extraction conditions (Maache *et al.*, 2025). Thus, some infusions shown in Table 1 presented TPC quantities comparable to those reported by Maache *et al.* (2025), reinforcing its potential as a natural source of bioactive compounds when water is used as solvent.

An alternative way to express antioxidant capacity and phenolic compound content is to report them in relation to a usual consumption portion, such as 200 mL, providing a more practical perspective on its benefits based on a possible daily consumption, as shown in Table 2. However, it is worth noting that the amount of plant indicated by manufacturers for preparing *S. officinalis* infusion is not standardized and specific, which may result in variations in the concentration of these compounds in the extracts and, consequently, in the antioxidant activity of the infusion when consumed.

A study performed by Pérez-Jiménez *et al*. (2008), determined the antioxidant capacity in beverages present in the Mediterranean diet measured by ABTS$^{\bullet+}$, where the value for tea was 6,308 μmol Trolox/L. The infusion presented 1,261.6 μmol of Trolox equivalents in 200 mL. Therefore, it can be considered that a diet that includes the consumption of sage infusions can provide a large part of this value per serving.

A similar study reported that TPC values for *S. officinalis* infusion in different growing areas ranged from 13.7 to 72.6 mg GAE/100 mL, that is, 27.4 to 145.2 mg GAE/200 mL (Rusaczonek *et al.* 2010). Thus, most of our results are within the range reported by the authors. However, five samples presented higher values (CS1/2>CS6/1>CS3/1> CS1/3,CS2/1).

Skotti *et al* (2014) reported values of 328 μmol Trolox/200 mL for DPPH$^{\bullet}$, and 326 μmol Trolox/200 mL for ABTS$^{\bullet+}$ for infusions of *S. officinalis*. These values are much lower than what was found in our study, mainly because it only involve leaves.

It is worth highlighting that data on commercial *S. officinalis* infusions are scarce. Also, the combination of plant stem and leaves represents a higher limitation for direct comparisons with the results of this study, since most of the available studies evaluate the leaves or other parts isolated. However, the data obtained are of great relevance, as they reflect conditions closer to those used in consumption, considering that the infusion is often prepared using the entirety content of the packages. Thus, our results contribute to a more realistic understanding of the chemical and functional characteristics of the consumed infusion.

The results show that *S. officinalis* is an important source of bioactive compounds and that infusions prepared with a mixture of leaves and stems offer significant benefits to human health, highlighting their potential for therapeutic applications. However, significant differences were found between the evaluated samples, which may be related to the origin of the plant, form of processing, and storage time, highlighting the importance of a comprehensive evaluation of the

Table 2 – Total phenolic content (TPC), and antioxidant capacity of infusions of commercial and botanically identified samples of *S. officinalis* in 200 mL (cup).

Samples	Assays			
	TPC [1]	DPPH$^{\bullet}$ [2]	ABTS$^{\bullet+}$ [2]	FRAP [3]
1 (CS1/1)	102 ± 2^{g}	722 ± 14^{g}	702 ± 48^{f}	2318 ± 97^{f}
2 (CS1/2)	216 ± 1^{a}	1486 ± 49^{a}	1207 ± 46^{b}	4457 ± 73^{a}
3 (CS1/3)	163 ± 4^{c}	1079 ± 13^{d,e}	1003 ± 46^{c}	3219 ± 93^{d}
4 (CS1/4)	111 ± 1^{f}	646 ± 21^{h}	737 ± 49^{e,f}	736 ± 36^{h}
5 (CS2/1)	163 ± 3^{c}	1068 ± 16^{e}	1155 ± 36^{b}	3193 ± 152^{d}
6 (CS3/1)	202 ± 3^{b}	1356 ± 30^{b}	1413 ± 147^{a}	4277 ± 47^{b}
7 (CS3/2)	132 ± 8^{d}	579 ± 19^{c}	1018 ± 56^{c}	3936 ± 72^{c}
8 (CS4/1)	94 ± 2^{h}	629 ± 37^{h}	654 ± 21^{f}	2175 ± 61^{f}
9 (CS5/1)	109 ± 1^{f,g}	847 ± 28^{f}	849 ± 29^{d,e}	2710 ± 104^{e}
10 (CS6/1)	215 ± 4^{a}	1118 ± 31^{c,d}	862 ± 57^{d}	4231 ± 215^{b}
11 (Spring)	91 ± 3^{h}	483 ± 18^{i}	396 ± 17^{h}	2176 ± 30^{f}
12 (Summer)	47 ± 0^{i}	137 ± 3^{j}	267 ± 14^{i}	541 ± 8^{h}
13 (Autumn)	119 ± 2^{e}	469 ± 24^{i}	541 ± 30^{g}	1767 ± 31^{g}
14 (Winter)	53 ± 1^{i}	141 ± 4^{j}	190 ± 20^{i}	690 ± 24^{h}

Legend: different lowercase letters in the same column indicate that the results are statistically different ($p < 0.05$). 1 = results expressed in mg GAE/200 mL. 2 = results expressed in μmol Trolox/200 mL. 3 = results expressed in μmol Fe^{2+}/200 mL. Sample 11 to 14: Botanically identified samples (BS); Commercial samples (CS); Commercial sample brand 1/batch 1 (CS1/1). Results expressed on a dry basis. Source: Prepared by the author, 2025.

chemical composition of the plant from different origins, which would make it possible to establish chemical markers, as an instrument for higher regulation of the medicinal plants market.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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