Advanced Mass Spectrometry Profiling of Phenolic and Minerals Compounds in Herbal Beverages

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ABSTRACT.

The global pandemic of COVID-19 has led to an increased interest in herbal infusions as natural remedies since 2020. This has also heightened the need for controlling the composition of these dietary supplements to ensure consumer health and prevent food fraud. In the present work, various mass spectrometry techniques were used to analyze the organic and inorganic composition of 23 herbal infusion samples. UHPLC-ESI-QTOF-MS was used to determine target, suspect, and nontarget polyphenolic compounds. Thus, 8 phenolic compounds were identified in the target analysis and additionally, 80 extra-compounds were identified through suspect and nontargeted screening. ICP-MS was used to monitor the metals released during tea leaf infusion, providing a complete mineral composition of each sample. Principal Component Analysis (PCA) and Discriminant Analysis (DA) were utilized to identify relevant compounds for differentiating and grouping the samples, thus serving as specific markers to detect potential food fraud.

1. Introduction

Tea (*Camelia sinensis*) and other herbal infusions have been used worldwide for centuries as diet complementary food due to their health-promoting properties (Jonas et al., 2013). Although, historically, the use of herbal infusions has had a greater impact in Eastern cultures, in recent years there has been an international resurgence of interest in plant-based products (X. Xu et al., 2021). The late increasing interest in dietary supplements was in part a consequence of the COVID-19 pandemic because patients have been looking for alternative and self-care practices to relieve the symptoms of this disease (Paudyal et al., 2022). The COVID-19 pandemic led to an enormous increase in sales of dietary supplements and nutraceuticals in early 2020, in the United States there was a 44 % ($435 million) rise in sales during the first wave of the pandemic (Lordan, 2021). Tea has garnered attention for its potential benefits in combating the associated symptoms of COVID-19, since certain antioxidant compounds, such as catechins, and flavonoids, which possess anti-inflammatory and antiviral properties present in tea leaves have potential effects on the immune system and overall well-being (Tallei et al., 2021). For example, epigallocatechin gallate (EGCG) which is a catechin found abundantly in green tea, has shown effects in inhibiting viral replication and preventing viral attachment to host cells (El-Missiry et al., 2021). Additionally, black tea, white tea, and herbal infusions like chamomile, ginger, and elderberry have also gained attention for their immune-boosting properties, which may help support the body's natural defense mechanisms during viral infections (Alamgir, 2018).

It is well known that most of the health-promoting intake of herbal infusions is correlated to the antioxidant activities of phytochemical compounds, such as polyphenols (Kang et al., 2020; Pinto et al., 2020). Polyphenols, which are critical secondary metabolites present in many plants (Zhang et al., 2021), are phenolic systems characterized by phenyl rings and one or more hydroxyl substituents (Singla et al., 2019). Polyphenols represent the largest group of phytochemical compounds,

there are more than 10,000 known polyphenols, including phenolic acids, flavonoids, and tannins (López-Fernández et al., 2020). The type and concentration of polyphenols in herbal infusions will change depending on several factors, such as the herb's specie, cultivars' geographical locations, climate conditions, or cultivation process (Ali et al., 2021; Durazzo et al., 2019; Yi et al., 2019). To control the polyphenolic profiles of tea and other herbs, it is important to develop selective, sensitive, and versatile analytical methods. However, generating comprehensive profiling of these compounds is difficult due to their complex structure: phenolic compounds can be found in simple or highly polymerized structures and commercial standards for proper identification of these bioactive compounds are unavailable (Ali et al., 2021; López-Fernández et al., 2020). Liquid chromatography (LC) coupled with triple quadrupole time-of-flight (QTOF) mass spectrometry is an advanced analytical technique able to solve this problem. Identification and characterization of polyphenols can be achieved by LC-QTOF analysis, in both target and non-target approaches (Chou et al., 2021; Feng et al., 2020; Gu et al., 2019; Ma et al., 2019). However, LC-QTOF could result in the generation of huge information that could be difficult to process. For this reason, it is common to use chemometric tools to interpret and introduce the most significant meaning to this information (Drira et al., 2020; Kalogiouri et al., 2016; Tzachristas et al., 2021).

In addition to organic compounds, plants also absorbed minerals from soil and air during their growth process. Then, all these minerals are introduced into the human diet and influence human health. Tea and other herbal infusions can be an adequate source of potassium, zinc, magnesium, calcium, and copper in the diet (Atasoy et al., 2019; Klepacka et al., 2021). Nevertheless, the concentration of these micronutrients in the body must remain constant at a certain level to avoid health disorders such as osteoporosis or hypertension (Karak et al., 2017; Yi et al., 2019). Inductively coupled plasma mass spectrometry (ICP-MS) is a sensitive and flexible analytical technique that allows determining the presence of almost any element at trace concentrations in tea samples (Atasoy et al., 2019; Picó, 2015).

In the present work, polyphenolic and mineral profiles of several herbal infusions (green tea, black tea, rooibos, and other infusions) have been determined using mass spectrometry techniques (ICP-MS and UHPLC-MS). The obtained information can be used to characterize the composition and concentration of phenolic compounds and essential minerals, such as manganese, potassium, and calcium, present in different herbal infusions. In addition, chemometric tools have been applied to identify possible representative compounds for each group of samples. This type of marker can be useful in rapid screenings, quality controls, or fraud detection. Overall, the study seeks to provide comprehensive information about various herbal infusions, as well as to explore the potential of the identified markers for assessing the authenticity, quality, and integrity of this type of dietary supplement products.

2. Materials and Methods

Standards and Reagents

Benzoic acid (ref. 141014.1210) and salicylic acid (ref. 141045.1210) were supplied by Panreac. Catechin (ref. C1788) and rosmarinic acid (ref. H37451.03) were supplied by Alfa Aesar. Cinnamic acid (ref. 96340) was supplied by Fulka. Gallic acid (ref. 447581000) was supplied by Acros Organics. Vanillic acid (ref. 94770) and vanillin (ref. V1104) were supplied by Sigma Aldrich. ESI Positive Calibration Solution for X500B (ref. 5049910) was purchased from AB SCIEX Spain S.L. Formic acid Optima® for LC/MS (99.5%, ref. A117-50) was purchased from Thermo Fisher Scientific. Methanol for UHPLC (99.9%, Ref. 83638320) was supplied by VWR.

Nitric Acid for Trace Metal analysis (ppb) (69%, ref. 721037.911) and Hydrochloric Acid for Trace Metal analysis (ppb) (35%, ref. 721019.011) were supplied by Panreac. Quality Control Standard 26: Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Si, Ti, Tl, V, Zn (ref. QCS-26-100) was supplied by High-Purity Standards. Certipur® Mercury standard solution (1000 mg/L, ref. 1.70226.0100) was supplied by Merck. Internal Standard Mix: Bi, Ge, In, Li6, Sc, Tb, and Y (10 mg/L, ref. N9303832) was supplied by PerkinElmer.

Sample Preparation

Twenty-three infusion samples were selected for this study: 6 green teas (*Camellia sinensis*), 7 black tea (*Camellia sinensis*), 4 rooibos (*Aspalathus linearis*) infusions and 6 infusions with no tea or rooibos leaves. Samples were prepared by weighing 1.0 g of dry leaves and adding 100 mL of boiling Milli-Q water. Each sample was left to infuse for 3 min and filtered through 0.22 µm nylon syringe filters (Filter-Lab).

Samples for UHPLC-QTOF analysis were prepared by diluting infusion samples with Milli-Q water (1 mL/10 mL or 1 mL/100 mL as required) before injection.

Samples for ICP-MS analysis were prepared by diluting infusion samples (1 mL/10 mL) with an acidic solution (1 % HNO_3/ 0.5 % HCl). Internal Standard Mix solution was added before analysis to obtain a final concentration of 50 µg/L of each element.

UHPLC-QTOF Analysis

Polyphenol quantification on herbal infusion samples was conducted by external calibration using benzoic acid, salicylic acid, catechin, rosmarinic acid, cinnamic acid, gallic acid, vanillic acid, and vanillin as standards. A mixed stock solution at a concentration of 100 mg/L of each compound in Milli-Q water was prepared. Several dilutions were conducted to obtain a calibration curve from 0.1 mg/L to 5.0 mg/L of each compound.

Instrumental determination was done using a UHPLC ExionLC™ AD System (AB Sciex, USA) coupled with an X500B QTOF System (MS/MS) (AB Sciex, USA) with electrospray ionization (ESI) in negative mode. Chromatographic separation was done using a Kinetex EVO C18 Column (100 × 2.1 mm id, 2.6 µm particle size) from Phenomenex. Mobile phase A was 0.1% formic acid in Milli-Q water and B was methanol. A linear gradient program at a flow rate of 0.5 mL/min was used as follows: 0–15 min, from 0 to 20% (B); 15–25 min, from 20 to 40% (B); 25–35 min, from 40 to 60% (B); 35–40 min, from 60 to 100% (B); then 5 min to 100% (B) and back to 10% (B) for 5 min. The injection volume was set at 10 µL.

ESI source parameters: spray voltage, −4500 V; source temperature, 400 ◦C; nebulizer and auxiliary gas flow (N_2), 50 psi and 50 psi; curtain gas flow (N_2), 45 psi. Mass spectrometry analyses were performed applying a data-independent scan experiment (SWATH®) which uses a combination of a full-scan TOF spectrum (100- 1000 Da; declustering potential (DP), -70 V; collision energy (CE), -5 V) with TOF MS/MS fragmentation using fixed Q1 transmission windows. In this experiment 10 fixed SWATH windows of 100 Da were used to span across the precursor range of 100-1000 Da (DP, -70 V; CE, -35 ± 15 V). Data were processed using SCIEX OS 2.2 acquisition software.

ICP-MS Analysis

Semi-quantitative Elemental analysis on infusion samples was conducted with the external standard method by using a single calibration standard and a blank solution (1 % HNO3/ 0.5 % HCl). A stock solution of Multielement Standard (Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Si, Ti, Tl, V, Zn) and Mercury Single Standard was prepared at a concentration of 1 mg/L for each element. The stock solution was diluted to obtain a concentration of 50 µg/L and Internal Standard Mix solution was added before analysis to obtain a final concentration of 50 µg/L of each element.

Instrumental determination was done using a NexION® 5000 Multi-Quadrupole ICP-MS (PerkinElmer, Waltham, USA). The sample introduction system consists of a PFA nebulizer and a glass cyclonic spray chamber. The ICP-MS is equipped with a triple cone interface, where potential can be applied to reduce the amount of ion-beam that enters the quadrupole deflector (focusing). The instrument is also equipped with two quadrupoles to guide the ions generated in the plasma (MS/MS) and another quadrupole used, in this work, as a collision cell (KED mode). In this work, Helium gas (99,999 %, Linde plc) was used as inert gas for the KED mode. The RF power was 1600 W and the plasma gas flow was 16 L/min.

Chemometric Determinations

Statistical analyses were applied to group analyzed samples into different clusters according to their polyphenol and mineral profiles. Since the selected samples can be classified into pre-established groups (green tea, black tea, rooibos, and other herbal infusions), a supervised analysis such as discriminant analysis (DA) can be performed. DA was applied to the mineral composition results after standardization. In addition, MarkerView™ Software (SCIEX, Version 1.3.1) was used to perform further statistical analysis on differences in the selected samples on the LC-MS results. This software combines principal component analysis (PCA) with discriminant analysis (DA), considering all the information in the raw data from a single sample injection. Thus, MarkerView™ Software provides accurate results in different analyses and sample clustering. In addition, a SCIEX high-resolution MS/MS database with more than 900 natural products has been accessed. This, in combination with primary exact mass numbers and isotope distribution, led to compound identifications and differential analysis. 3D graphical representations were performed using Minitab® Software (2021, Version 20.3).

3. Results and discussion

3.1. Target and Suspect Analysis of Polyphenols

Chromatographic separation and full-spectrum acquisition at accurate mass, provided by UHPLC-QTOF analysis, enable the investigation of target, suspect, and non-target compounds (Hernández et al., 2012). First, quantitative targeted

analysis with reference standards was performed. Therefore, to quantify polyphenols in tea and other herbal infusions, a standard solution of 1 mg/L of benzoic acid, salicylic acid, catechin, rosmarinic acid, cinnamic acid, gallic acid, vanillic acid, and vanillin was analyzed (Supporting Information, Figure S1). Linear range with its determination coefficient (R^2), the limit of detection (LOD), and the limit of quantification (LOQ) were determined from calibration curves for each standard. Results are shown in Table S1 in the Supporting Information. LOD was calculated as the analyte concentration at 3 times the signal-to-noise ratio (S/N) of the most diluted standard, whereas LOQ was the analyte concentration at 10 times the S/N (Uhrovčík, 2014). The obtained R^2 values are very close to 1, indicating a strong correlation between concentration and instrumental response for each calibration curve (AOAC International, n.d.). The linear range represents the concentration interval within which results can be obtained with sufficient precision, accuracy, and reliability. The concentration of benzoic acid, salicylic acid, catechin, rosmarinic acid, cinnamic acid, gallic acid, vanillic acid, and vanillin in the 23 selected herbal infusions was determined by external standard calibration. Total Ion Chromatograms (TIC) for each type of samples can be seen in Figure S2 on the Supporting Information. Results are shown in Table 1.

Table 1. Determination of benzoic acid (BA), salicylic acid (SA), catechin (C), rosmarinic acid (RA), cinnamic acid (CA), gallic acid (GA), vanillic acid (VA), and vanillin (V) in 23 tea and infusion samples using UHPLC-QTOF method.

Sample	*[BA] ± s / μg·g^{-1}*	*[SA] ± s / μg·g^{-1}*	*[C] ± s / μg·g^{-1}*	*[RA] ± s / μg·g^{-1}*	*[CA] ± s / μg·g^{-1}*	*[GA] ± s / μg·g^{-1}*	*[VA] ± s / μg·g^{-1}*	*[V] ± s / μg·g^{-1}*
S1	1.6 ± 0.4	6.5 ± 0,.6	N/A	< 1.1	2.2 ± 0.4	N/A	7.9 ± 0.4	1.4 ± 0.3
S2	5.0 ± 0.5	23.2 ± 1.6	50.8 ± 9.9	103 ± 11	12.4 ± 1.9	294 ± 39	8.1 ± 1.2	3.3 ± 0.7
S3	6.0 ± 1.3	26.5 ± 2.2	N/A	4029 ± 531	1.8 ± 0.3	5.8 ± 0.1	27.1 ± 4.5	4.1 ± 0.7
S4	N/A	34.8 ± 1.0	377 ± 72	1.9 ± 0.4	2.1 ± 0.4	752 ± 123	8.9 ± 1.6	35.7 ± 3.4
S5	N/A	12.9 ± 1.2	87.7 ± 9.3	1072 ± 145	2.2 ± 0.3	717 ± 124	9.6 ± 0.8	7.4 ± 1.2
S6	N/A	22.2 ± 0.9	N/A	1.8 ± 0.3	N/A	N/A	5.5 ± 1.1	3.6 ± 0.8
S7	21.2 ± 4.3	30.2 ± 1.6	< 1.3	486 ± 64	22.6 ± 4.2	10.7 ± 1.4	65.4 ± 7.6	14.8 ± 2.9
S8	45.7 ± 3.5	8.8 ± 1.6	< 1.3	1644 ± 141	99.3 ± 20.1	N/A	18.8 ± 2.9	7.6 ± 1.6
S9	4.7 ± 1.0	13.2 ± 0.8	57.2 ± 7.3	3.2 ± 0.5	N/A	503 ± 78	11.8 ± 2.1	1313 ± 175
S10	13.4 ± 1.7	34.8 ± 0.9	28.1 ± 5.9	3.4 ± 0.4	59.8 ± 11.9	987 ± 154	19.1 ± 2.7	31.1 ± 4.5
S11	23.3 ± 3.9	6.0 ± 0.7	N/A	< 1.1	2.0 ± 0.3	N/A	33.9 ± 2.2	< 1.1
S12	18.3 ± 3.1	23.4 ± 3.3	41.0 ± 6.2	< 1.1	49.7 ± 9.3	1241 ± 235	17.8 ± 3.4	36.1 ± 7.1
S13	11.7 ± 1.9	1.4 ± 0.2	N/A	< 1.1	35.8 ± 6.2	3.9 ± 0.8	18.1 ± 3.7	51.2 ± 5.9
S14	N/A	12.4 ± 1.3	85.0 ± 12.6	112 ± 15	N/A	265 ± 48	13.7 ± 1.8	7.9 ± 1.5
S15	17.1 ± 3.4	4.5 ± 0.5	N/A	123 ± 20	81.6 ± 14.0	< 1.2	9.6 ± 1.5	7.7 ± 1.3
S16	32.0 ± 4.7	24.9 ± 3.3	1.5 ± 0.2	2.1 ± 0.4	61.1 ± 9.5	62.3 ± 9.6	57.0 ± 9.0	14.3 ± 2.5
S17	10.5 ± 2.0	11.4 ± 0.9	< 1.3	5864 ± 714	27.6 ± 2.6	2.2 ± 0.7	11.9 ± 2.3	1.4 ± 0.1
S18	30.2 ± 6.2	16.6 ± 1.1	< 1.3	3.3 ± 0.7	13.8 ± 3.1	79.3 ± 9.5	7.6 ± 1.5	< 1.1
S19	25.5 ± 2.4	24.8 ± 1.6	63.5 ± 9.3	< 1.1	225 ± 24	642 ± 99	18.9 ± 4.0	36.9 ± 5.3
S20	2.9 ± 0.4	6.7 ± 1.1	87.2 ± 9.5	< 1.1	14.7 ± 1.6	152 ± 27	3.4 ± 0.5	8.2 ± 1.7
S21	10.7 ± 1.8	27.8 ± 1.7	21.5 ± 3.3	< 1.1	2.8 ± 0.3	1416 ± 243	15.2 ± 3.1	1.8 ± 0.2

S22	16.9 ± 3.5	29.6 ± 1.6	6.7 ± 1.0	< 1.1	168 ± 11	413 ± 67	17.4 ± 3.4	2135 ± 314
S23	3.4 ± 0.7	11.6 ± 1.1	70.7 ± 9.3	171 ± 33	N/A	480 ± 89	6.0 ± 1.1	3.7 ± 0.6

N/A - not applicable as no chromatographic peak is detected
< n – below the LOQ

Table 1 shows very different polyphenol concentrations for the 23 studied samples. There is wide variability in the obtained results. Despite the difference in concentrations, salicylic acid, vanillic acid, and vanillin are always present in every sample. In some cases, such as rosmarinic acid and gallic acid, determined concentrations are in extremes: either among the upper concentration limit or below the limit of quantification. Moreover, ten of the studied samples (S2, S7, S10, S16, S17, S18, S19, S20, S21, and S22) contain, at different concentrations, all the 8 evaluated polyphenols.

To further characterize the polyphenolic profile of the selected 23 herbal infusion samples, a suspect compounds screening was performed. To achieve this goal, in-depth bibliographic research was carried out to identify polyphenolic compounds previously reported in tea and other herbal infusions. This resulted in a list of 61 target compounds, including their accurate mass information. The suspect screening was performed on the SWATH® results, establishing the following criteria for identification: S/N > 10, chromatographic signal 10 times higher in the sample than in the control, a mass error of less than 5 ppm, and isotope ratio difference lower than 10%. Furthermore, the MS/MS spectra of the candidates were compared with a SCIEX Natural Products database. A fit greater than 80% and the presence of at least two characteristic MS/MS fragments (mass error < 5 ppm) were used to strengthen the identification of tentative candidates. The obtained results can be seen in Table S2 in the Supporting Information. A total of 59 polyphenolic compounds out of 61 suspected targets were determined in the 23 herbal infusion samples. In the context of suspect analysis, it is important to establish criteria for compound annotation and identification. Thus, the identified compounds were classified into different confidence levels based on the international Metabolomics Standards Initiative (MSI) guidelines (Campos-Mañas et al., 2019). Identified compounds (level A) are those with the highest level of confidence and required matching to authentic standards. Then, the confidence level decreases from putatively identified compounds (level B, medium) to tentatively annotated compounds (level C, low), as poorer the collected information. The classification of polyphenolic compounds resulting from the targeted and suspect analysis can be found in Table S3 of the Supporting Information. In the present work, only 8 compounds were fully identified, classified in level A, corresponding to the target compounds (benzoic acid, salicylic acid, catechin, rosmarinic acid, cinnamic acid, gallic acid, vanillic acid, and vanillin).

3.2. Non-Target Analysis of Polyphenols

An untargeted analysis was conducted to further characterize the polyphenolic profile of each of the 23 samples. This analysis was carried out in negative ionization mode with the SWATH® acquisition method. To tentatively identify the unknown compounds detected from the non-target screening, several conditions must be accomplished. First, it must be a coincidence in retention time for a proposed compound in all the samples. Secondly, SCIEX OS can provide a list of chemical formulas based on the isotopic profile. Moreover, the mass error between theoretical m/z and observed m/z must be lower than ± 10 ppm. Finally, obtained MS/MS spectrum must be confirmed by comparison against the bibliographic MS/MS spectrum (SCIEX Natural Products database). In other words, there must be an agreement between exact mass, isotopic profile, and the fragments obtained by MS/MS. Obtained results are shown in Table S4 in the Supporting Information. Thus, following a non-targeted screening, 29 extra compounds have been tentatively identified in the analyzed samples. In all the cases, the mass error between observed and expected m/z values is lower than 3 ppm (Schulze et al., 2020).

Using targeted, suspect, and non-targeted analysis, it is possible to obtain a polyphenolic profile of the different analyzed samples. In addition, by applying chemometric tools to the raw data from UHPLC-MS analysis, it is possible to group the samples and find out characteristic compounds. This kind of information is useful in nutraceutical applications and the identification of possible food frauds (Miaw et al., 2022; Rovira et al., 2022).

In the present work, the 23 analyzed samples can be pre-grouped depending on the infusion type, as follows:

- Green tea: S4, S5, S9, S14, S20, and S23
- Black tea: S2, S8, S10, S12, S19, S21, and S22
- Rooibos: S3, S7, S16, and S17

- Other infusions: S1, S6, S11, S13, S15, and S18

Since pre-groups can be defined, Minitab software was used to perform a discriminant analysis (DA) using quantifications of benzoic acid, salicylic acid, catechin, rosmarinic acid, cinnamic acid, gallic acid, vanillic acid, and vanillin. Thus, it is expected to observe clustering trends and whether pre-established theoretical clusters are met. DA requires two assumptions: quantitative predictable variables and no correlation between variables (Henrion & Henrion, 2006). The first criterion is met as values used are concentrations. The second criterion had to be evaluated by calculating the correlation coefficients (r) between variables (see Figure S3, Supporting Information). In all the cases, the obtained r values are lower than ± 0.7, indicating a non-strong linear correlation between variables (Ratner, 2009). Thus, DA can be applied. However, tea and other herbal infusion studied samples have complex polyphenolic profiles, as could be deduced from the suspect and non-targeted analyses. For this reason, the eight selected variables are not enough representative to observe clustering trends and misclassifications may occur. To overcome this problem, MarkerView software was used, allowing combined supervised and unsupervised techniques like DA and principal component analysis (PCA). MarkerView allows the exploration of statistical correlations with direct connections using the entire raw data from a UHPLC-QTOF injection, where approximately 5.000 peaks are considered. Thus, more meaningful relationships can be achieved than just using 8 concentration variables. Hence, a PCA-DA was applied to the polyphenolic compounds grouped by herbal infusion type (see Figure 1).

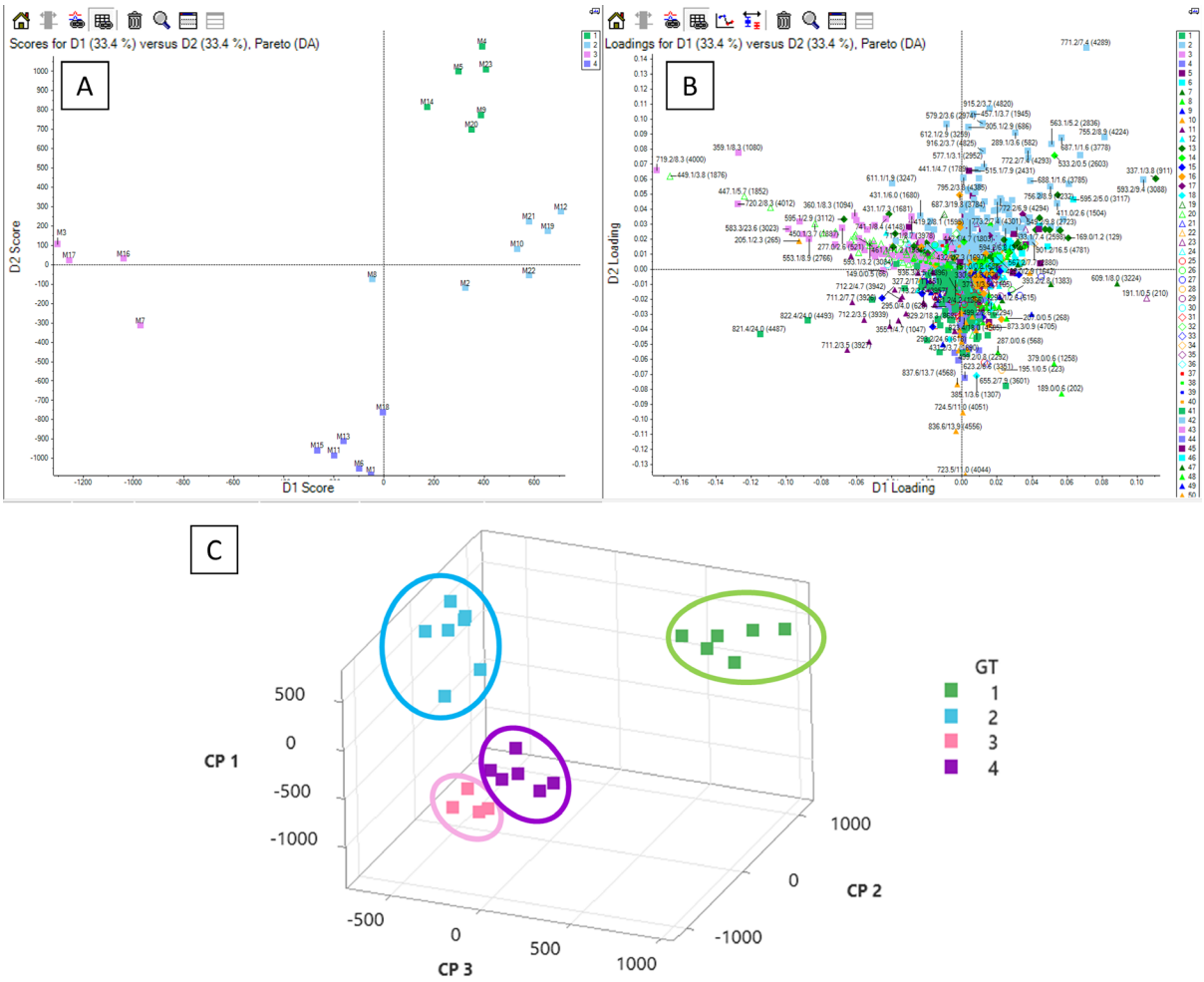

Figure 1. Principal component analysis coupled with discriminant analysis (PCA-DA): the Scores plot shows groups and differences among the samples (A) and the Loadings Plot (B) reflects the variables that are causing the separation (those with higher loadings values are generally more significant for the separation). In addition, a 3D representation considering three principal components was obtained by processing the data obtained from MarkerView with Minitab (C).

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In Figure 1.A, the first two principal components explain 66.8% of the variance (CP1: 33.4%; CP2: 33.4%), while considering the third principal component (CP3: 33.1%) allows for explaining a total variance of 99.9% using all variables from the raw data (approximately 5,000 variables). Figure 1.B represents the projection of the variables regarding the loadings of the variables on the two principal components. Since MarkerView does not allow the representation of the information of 3 principal components at a time, Minitab was used to obtain 3D graphics and to diminish the possible loss of information. The distribution of theoretical groups (GT) considering the three principal components can be seen in Figure 1.C. Clear clustering trends can be observed, with GT1 (green tea) scoring positively in all three components, GT2 (black tea) scoring positively in the first two components and negatively in the third, and GT3 (rooibos) and GT4 (other infusions) scoring negatively in the first and third components and positively in the second.

Moreover, using MarkerView it was possible to identify the characteristic compounds of each group. This software provided a table with information on m/z, retention time, and the impact value that variables have on a selected CP. Consequently, the variable with the highest impact will be the most characteristic of each group. An example can be seen in Figure 2.

A

Index	Peak Name	m/z	Ret. Time	Impact
3248	611.1/2.9 (3248)	611.1414	2.87	2.306e-1
4820	915.2/3.7 (4820)	915.1641	3.68	2.053e-1
4000	719.2/8.3 (4000)	719.1615	8.33	2.017e-1
4289	771.2/7.4 (4289)	771.2007	7.42	1.997e-1
1876	449.1/3.8 (1876)	449.1093	3.84	1.866e-1
1945	457.1/3.7 (1945)	457.0785	3.68	1.841e-1
1080	359.1/8.3 (1080)	359.0774	8.33	1.669e-1
911	337.1/3.8 (911)	337.0941	3.82	1.626e-1
686	305.1/2.9 (686)	305.0670	2.87	1.615e-1

B

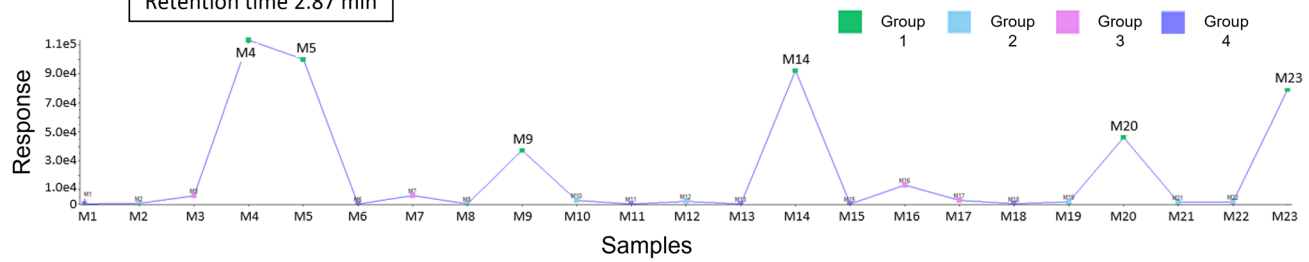

Figure 2. List of some variables with their associated m/z and retention times values ordered by impact factor over the sample group 1 (A) and the graphical representation of the impact factor of variable no. 3248 (m/z 611.1414 Da and retention time 2.87 min) over the 23 tea and other herbal infusion samples (B).

As can be seen in Figure 2.A, variables were ordered by their impact factor within each theoretical group. Then, by plotting the abundance of one variable across the samples it could be easily selected the more representative variable for each group. Figure 2.B highlights variable nº 3248 (m/z 611.1414 Da, RT 2.87 min) as the key differentiating factor for green tea samples. Based on this information, characteristic variables were identified for all four sample groups. Then, a non-targeted workflow analysis was applied to assign possible compounds to each variable. Figure 3 shows the extracted ion chromatogram for the m/z 611.1414 on the green tea sample S4, mass spectrum at 2.88 min, and the corresponding MS/MS spectrum.

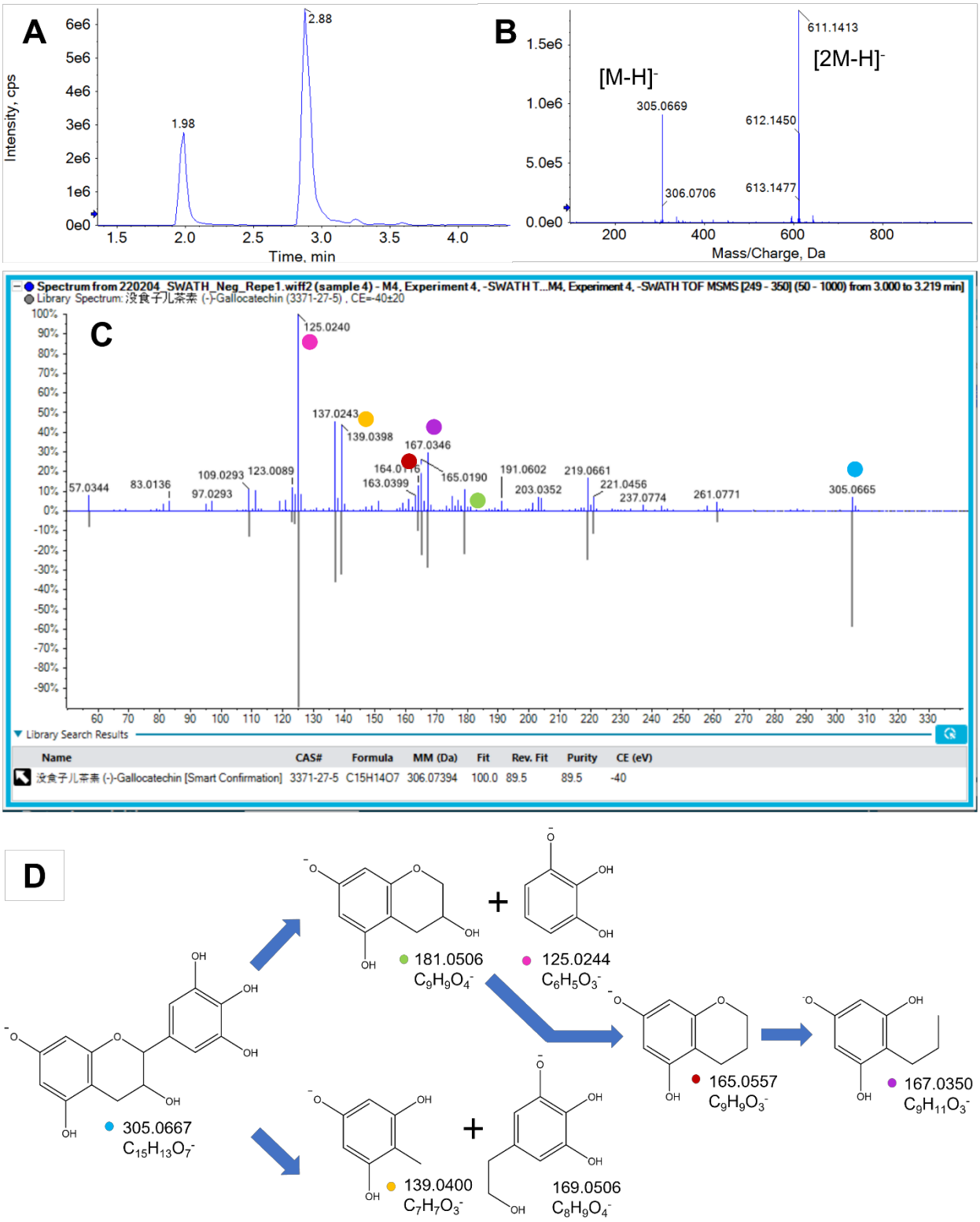

Figure 3. Extracted ion chromatogram (XIC) for the m/z 611.1414 on the green tea sample S4 (A), the TOF MS spectrum obtained at 2.879 min (B), and the corresponding MS/MS experimental spectrum when a CE = -35 ± 15 V was applied (blue line) compared with a

bibliographical spectrum from a SCIEX Natural Products database (grey line) (C). Process diagram for gallocatechin fragmentation deduction (D).

In Figure 3.A, two chromatographic peaks were observed from the ion extraction of m/z 611.1414 from tea gran sample S4, with retention times of 1.98 min and 2.88 min. The characteristic variable selected from MarkerView was set at 2.87 min, so the mass spectrum corresponding to this chromatographic peak was first analyzed (Figure 3.B). Two molecular ions were observed: the target m/z 611.1414 and another m/z 305.0670. Considering that the target m/z (611.1414) is twice the observed extra m/z (305.0670) and that the isotopic profiles indicating monocharged species, it is hypothesized that the target m/z is the dimer ($[2M-H]^-$) of the observed extra m/z, which is the actual molecular ion ($[M-H]^-$). Dimer formation of phenolic compounds in tea samples is well-documented, such as rosmarinic acid (dimer of caffeic acid) and ellagic acid (dimer of gallic acid) (Engelhardt, 2010; Tsao, 2010). Furthermore, a chemical formula can be proposed from the mass spectrum. The molecular ion $[M-H]^-$ was 305.0670 Da, resulting in an exact mass of 306.0739 Da. The chemical formula proposed for this exact mass is $C_{15}H_{14}O_7$. Futhermore, MS/MS spectra were compared with the SCIEX Natural Products database to find the most suitable polyphenol candidate (see Figure 3.C). A match was found with the compound Gallocatechin (chemical formula $C_{15}H_{14}O_7$, monoisotopic mass 306.0739 Da).

Nevertheless, green tea samples showed another chromatographic peak at 1.98 min with the same mass spectrum and MS/MS profile as the 2.87 min peak (see Figure S4, Supporting Information). This fact strengthens the hypothesis of the presence of isomers. The presence of epimers is proposed, which are diastereoisomers that differ in the configuration of a single stereogenic center and are common in polyphenols (J. Z. Xu et al., 2003). From the experimental MS/MS it was possible to identify one of these two compounds corresponded to gallocatechin, as previously said. Therefore, the other compound is probable to be epigallocatechin (J. Z. Xu et al., 2003). However, as no standards were available, it was not possible to confirm this hypothesis. Nevertheless, it was possible to estimate the RT based on the behavior of catechin (available standard) and take into account the information available in literature (Lee et al., 2014; J. Z. Xu et al., 2003). Thus, it was estimated that gallocatechin will elute first (1.98 min), followed by epigallocatechin (2.87 min). Therefore, the compound hiding under variable no 3248 and which is characteristic of the green tea group is most likely to be epigallocatechin. The same deductive process was applied to identify characteristic compounds for each group of samples. Results are shown in Table 2.

Table 2. Characteristic candidate compounds specifics of each sample group (green tea, black tea, rooibos, and other herbal infusions).

Group	No. variable	Impact value	*m/z*	RT /min	Proposed compound
Green Tea	3248	0.23	611.1414 [a]	2.87	Epigallocatechin or gallocatechin ($C_{15}H_{14}O_7$)
	4820	0.21	915.1641 [a]	3.68	Epigallocatechin gallate or gallocatechin gallate ($C_{22}H_{18}O_{11}$)
Black tea	595	0.04	291.0153	3.68	Brevifolincarboxilic acid ($C_{13}H_8O_8$)
	663	0.04	300.9991	7.85	Ellagic acid ($C_{14}H_6O_8$)
Rooibos	1876	0.19	449.1093	3.84	Astilbin or Smitilbin ($C_{21}H_{22}O_{11}$)
	438	0.07	255.0512	1.92	Piscidic acid ($C_{11}H_2O_7$)
Other herbal infusions	1307	0.08	385.0783	3.56	2-(E)-feruloyl-D-galactaric acid ($C_{16}H_{18}O_{11}$)

[a] The m/z value corresponds to the dimer of the compound.

As shown in Table 2, it was possible to tentatively identify one or two characteristic compounds for each group of samples. Epigallocatechin or gallocatechin and epigallocatechin gallate or gallocatechin gallate were proposed as

characteristic markers of green tea samples. Catechins represent approximately 30% of the green tea leaf's dry weight and are considered the main responsible for its health benefits, especially epigallocatechin gallate (Ko et al., 2021; Sivanesan et al., 2022). For this reason, it could be expected that catechins are determinants in the classification of green tea samples.

Brevifolincarboxylic acid and ellagic acid were proposed for black tea samples. Black tea is obtained from the fermentation of green tea leaves. During this process, phenolic acids content increases as a consequence of the oxidation of phenols. Thus, the presence of compounds such as ellagic acid is usually higher in samples of black tea compared to green tea (Yang & Tomás-Barberán, 2019; Zhao et al., 2019).

For rooibos (*Aspalathus linearis*) samples, the proposed characteristic compounds were astilbin or smitilbin and piscidic acid. Other authors have reported that pisidic acid, a derivative of phenylpyruvic acid, is present in rooibos samples and it is also one of the components that most influence the classification of rooibos samples compared to other types of infusions (M.A. Stander et al., 2019; Maria A. Stander et al., 2017). Therefore, the results obtained in this work are in good agreement with the literature.

Finally, 2-(E)-feruloyl-D-galactaric acid was proposed as a characteristic marker for other herbal infusions. In this case, the analyzed infusions contained some kind of berries and citrical fruits in their formulation and 2-(E)-O-feruloyl-D-galactaric acid is present in this kind of fruit (Olivares-Caro et al., 2020; Santiago et al., 2022).

In some cases, specific compound determination was challenging due to epimers or indistinguishable isomers, such as variable no. 1876. Nevertheless, the 23 analyzed samples were successfully grouped according to theoretical groups. Furthermore, it has been possible to putatively determine at least one characteristic compound for each group, which potentially allows the classification of the samples. Therefore, it is possible to conclude that the statistical analyses applied in the present work (PCA-DA) allow the classification of the herbal tea samples according to the polyphenol profile they present, as this is characteristic of each group of samples.

3.3. Mineral Composition

Tea and other herbs contain minerals in their leaves that are extracted into hot drinks. These minerals and metals are consumed and have a direct effect on health. Therefore, it is necessary to control the presence and concentration of these compounds to prevent trace mineral imbalances and/or heavy metal poisoning. Tea leaves are well known to contain several elements such as potassium, manganese, selenium, boron, zinc, strontium, copper, fluoride, magnesium, calcium, and aluminium.

In this work, a semi-quantitative elemental ICP-MS analysis was applied to determine in a single acquisition the total inorganic composition of herbal infusion samples. By using ICP-MS and a semi-quantitative software package (TotalQuant in Syngistix for ICP-MS, PerkinElmer), an unknown sample can be analyzed for 81 elements in less than three minutes, providing information on the complete mass spectrum, typically within ± 25% of the quantitative values (Perkin Elmer, 2001; Pruszkowski, 2014). During TotalQuant analysis, it was assigned a response value to each element and spectral interpretation was performed automatically by the software; intensities were assigned to elements after correction for interferences on individual isotopes (Pruszkowski, 2014). Thus, the TotalQuant software was used to screen the elements present in the 23 selected samples. The mineral composition was assessed by extracting 1 g of herbal leaves in 100 mL of hot water. In this way, it was intended to simulate the consumption of a cup of infusion. Three replicates of each measure were carried out. Table 3.A and 3.B show the elements found in each sample and their concentrations.

Table 3.A. Mineral and heavy metal average concentrations ($mg \cdot L^{-1}$) from 3 measurement replicates in infusions of tea and other herbs ([Analyte]average ± 2s / $mg \cdot L^{-1}$).

Samples		Na	Mg	Al	Si	P	K	Ca	Mn	Rb
Green tea	***S4***	1.9 ± 0.2	4.8 ± 0.5	6.3 ± 0.3	0.5 ± 0.2	37.7 ± 2.6	107.7 ± 6.0	1.7 ± 0.4	2.3 ± 0.1	0.26 ± 0.07
	S5	7.0 ± 1.0	12.4 ± 1.3	4.4 ± 0.5	0.6 ± 0.1	57.6 ± 6.2	147.3 ± 21.4	4.6 ± 1.0	2.8 ± 0.1	0.33 ± 0.07
	S9	3.2 ± 0.6	9.6 ± 0.4	4.9 ± 0.3	0.7 ± 0.1	61.6 ± 9.7	109.1 ± 8.2	4.4 ± 0.5	2.4 ± 0.1	0.40 ± 0.12
	S14	7.2 ± 1.0	8.6 ± 0.9	4.5 ± 0.6	0.9 ± 0.1	43.5 ± 2.9	119.3 ± 14.1	1.1 ± 0.6	1.8 ± 0.1	0.67 ± 0.17
	S20	3.9 ± 2.7	2.3 ± 0.5	1.4 ± 0.3	0.2 ± 0.1	19.5 ± 5.9	56.1 ± 7.2	2.7 ± 0.3	0.79 ± 0.05	0.32 ± 0.07
	S23	5.2 ± 1.3	3.6 ± 0.7	1.3 ± 0.2	0.3 ± 0.1	30.3 ± 7.1	78.1 ± 11.4	0.10 ± 0.01	0.85 ± 0.01	0.54 ± 0.12
Black tea	***S2***	4.4 ± 1.1	22.2 ± 4.0	1.0 ± 0.2	0.4 ± 0.1	35.7 ± 12.3	131.8 ± 13.9	23.8 ± 1.8	3.0 ± 0.2	0.63 ± 0.12
	S8	32.4 ± 9.0	16.3 ± 2.8	BDL	0.6 ± 0.2	45.3 ± 5.0	126.0 ± 6.7	20.8 ± 2.3	0.16 ± 0.01	0.21 ± 0.06
	S10	12.9 ± 3.2	10.9 ± 0.9	1.9 ± 0.3	0.6 ± 0.1	71.8 ± 8.9	161.2 ± 6.3	BDL	0.92 ± 0.08	0.57 ± 0.13
	S12	4.9 ± 1.7	9.3 ± 0.9	1.9 ± 0.1	0.5 ± 0.1	50.3 ± 3.8	141.7 ± 15.6	1.1 ± 0.4	1.51 ± 0.06	0.51 ± 0.13
	S19	11.1 ± 4.0	7.2 ± 0.9	1.3 ± 0.1	0.4 ± 0.2	42.3 ± 5.3	114.5 ± 6	0.9 ± 0.2	0.72 ± 0.01	0.40 ± 0.09
	S21	6.3 ± 1.2	3.5 ± 0.2	2.8 ± 0.2	0.4 ± 0.1	58.1 ± 2.9	93.5 ± 8.1	0.9 ± 0.1	1.69 ± 0.05	0.95 ± 0.22
	S22	12.9 ± 1.9	8.4 ± 1.2	1.2 ± 0.3	0.4 ± 0.3	38.7 ± 7.9	96.3 ± 11.3	1.6 ± 0.1	1.08 ± 0.07	0.58 ± 0.14
Rooibos	***S3***	7.5 ± 2.4	11.7 ± 1.1	BDL	0.7 ± 0.1	41.2 ± 6.1	104.5 ± 8.3	18.9 ± 2.5	0.13 ± 0.01	0.12 ± 0.03
	S7	48.2 ± 4.6	12.8 ± 0.6	0.04 ± 0.01	0.4 ± 0.2	55.3 ± 7.0	122.3 ± 4.2	12.6 ± 0.6	0.26 ± 0.01	0.15 ± 0.03
	S16	21.6 ± 0.4	5.1 ± 0.2	BDL	0.2 ± 0.1	23.8 ± 2.4	34.0 ± 2.6	3.2 ± 0.1	0.15 ± 0.01	0.11 ± 0.03
	S17	15.6 ± 2.8	14.1 ± 0.9	BDL	0.6 ± 0.2	56.1 ± 9.4	143.7 ± 14.5	24.4 ± 2.3	0.09 ± 0.01	0.15 ± 0.03
Other herbs	***S1***	8.4 ± 1.8	3.3 ± 0.3	BDL	1.1 ± 0.1	5.5 ± 1.2	18.9 ± 1.8	14.0 ± 1.5	0.04 ± 0.01	0.02 ± 0.01
	S6	1.4 ± 0.2	13.1 ± 0.7	BDL	1.1 ± 0.1	35.8 ± 7.8	98.1 ± 11.1	24.1 ± 1.6	0.09 ± 0.01	0.25 ± 0.06
	S11	2.4 ± 0.8	3.5 ± 0.2	BDL	BDL	18.6 ± 4.4	43.9 ± 3.1	8.2 ± 1.0	0.04 ± 0.01	0.08 ± 0.02
	S13	19.3 ± 3.2	13.5 ± 1.2	BDL	1.7 ± 0.4	44.6 ± 6.7	173.3 ± 30.5	4.3 ± 1.6	0.26 ± 0.05	0.19 ± 0.03
	S15	21.3 ± 1.2	10.2 ± 0.3	BDL	3.7 ± 0.3	24.0 ± 2.5	77.0 ± 8.1	17.0 ± 0.4	0.13 ± 0.01	0.25 ± 0.06
	S18	13.4 ± 3.8	23.4 ± 2.4	0.05 ± 0.02	0.6 ± 0.2	88.1 ± 6.6	174.5 ± 9.3	45.7 ± 1.3	1.33 ± 0.04	0.24 ± 0.05

BDL, Below Detection Limit (0.04 mg/L, calculated using 3σ approach)

Table 3.B. Mineral and heavy metal average concentrations ($\mu g \cdot L^{-1}$) from 3 measurement replicates in infusions of tea and other herbs ([Analyte]average ± 2s /$\mu g \cdot L^{-1}$)

Samples		Fe	Ni	Cu	Zn	As	Sr	Cd	Ba	Hg	Pb
Green tea	***S4***	BDL	74.6 ± 5.3	71.1 ± 0.3	103.8 ± 7.6	BDL	23.8 ± 1.9	BDL	16.7 ± 0.3	BDL	BDL
	S5	12.3 ± 3.0	48.1 ± 2.7	66.0 ± 3.4	122.7 ± 6.4	BDL	47.7 ± 10.1	BDL	27.4 ± 1.7	BDL	BDL
	S9	BDL	65.7 ± 3.4	100.1 ± 4.9	119.3 ± 8.0	BDL	51.1 ± 15.5	BDL	26.7 ± 0.3	BDL	BDL
	S14	BDL	36.7 ± 1.8	61.8 ± 2.9	114.9 ± 4.9	BDL	17.5 ± 7.1	BDL	21.7 ± 1.6	BDL	BDL
	S20	BDL	10.2 ± 0.5	24.5 ± 1.0	67.8 ± 5.2	BDL	27.7 ± 7.2	BDL	34.0 ± 2.3	BDL	BDL
	S23	BDL	32.4 ± 1.7	54.6 ± 1.6	103.2 ± 2.7	BDL	BDL	BDL	8.3 ± 0.3	BDL	BDL
Black tea	***S2***	31.0 ± 12.9	18.0 ± 1.0	27.5 ± 1.0	217.9 ± 14.1	BDL	269.6 ± 74.7	BDL	153.6 ± 10.7	BDL	BDL
	S8	7.2 ± 1.0	14.2 ± 0.5	58.9 ± 1.7	87.7 ± 3.1	BDL	223.9 ± 62.4	BDL	30.9 ± 0.8	BDL	BDL
	S10	30.8 ± 12.6	20.8 ± 1.8	85.2 ± 2.6	155.8 ± 17.6	BDL	BDL	BDL	21.8 ± 1.3	BDL	BDL
	S12	56.7 ± 15.2	20.1 ± 1.8	55.7 ± 0.9	131.2 ± 2.6	BDL	11±3	BDL	16.7 ± 0.6	BDL	BDL
	S19	17.7 ± 11.8	27.6 ± 1.7	66.0 ± 2.0	123.7 ± 11.4	BDL	BDL	BDL	13.5 ± 0.3	BDL	BDL
	S21	40.1 ± 14.4	27.2 ± 0.5	57.9 ± 3.6	107.2 ± 3.9	BDL	23.5 ± 3.9	BDL	31.9 ± 1.2	BDL	BDL
	S22	18.7 ± 13.2	19.3 ± 0.9	53.3 ± 0.3	132.7 ± 10.9	BDL	13.2 ± 3.5	BDL	17.4 ± 0.3	BDL	BDL
Rooibos	***S3***	45.7 ± 16.8	12.0 ± 0.8	35.6 ± 2.1	125.6 ± 11.6	BDL	90.7 ± 21.5	BDL	44.3 ± 2.8	BDL	BDL
	S7	76.8 ± 7.0	23.5 ± 0.6	59.2 ± 0.3	89.4 ± 5.5	BDL	172.2 ± 40.6	BDL	19.0 ± 1.0	BDL	BDL
	S16	37.6 ± 15.4	BDL	22.7 ± 0.2	79.7 ± 4.4	BDL	47.2 ± 12.7	BDL	11.5 ± 0.7	BDL	BDL
	S17	11.1 ± 0.2	14.2 ± 1.6	59.9 ± 2.2	87.5 ± 2.6	BDL	169.9 ± 41.8	BDL	23.4 ± 1.2	BDL	BDL
Other herbs	***S1***	BDL	BDL	15.5 ± 1.0	107.2 ± 1.7	BDL	62.1 ± 15.5	BDL	18.1 ± 0.5	BDL	BDL
	S6	BDL	BDL	73.3 ± 2.5	69.6 ± 7.0	BDL	262.6 ± 53.7	BDL	149.7 ± 3.7	BDL	BDL
	S11	BDL	BDL	29.9 ± 2.1	189.5 ± 20.7	BDL	49.2 ± 12.5	BDL	12.5 ± 1.3	BDL	BDL
	S13	BDL	BDL	43.6 ± 4.5	76.8 ± 6.7	BDL	34.1 ± 6.1	BDL	31.8 ± 3.0	BDL	BDL
	S15	BDL	BDL	60.8 ± 3.3	84.4 ± 7.6	BDL	160.6 ± 35.6	BDL	26.3 ± 1.9	BDL	BDL
	S18	91.9 ± 32.4	18.7 ± 0.8	66.5 ± 2.0	215.0 ± 2.4	BDL	256.6 ± 66.1	BDL	95.4 ± 1.6	BDL	BDL

BDL, Below Detection Limit (3 µg/L, calculated using 3σ approach)

As can be seen in Table 3.A and 3.B, Na, Mg, Al, P, K, Ca, Mn, Fe, Ni, Cu, Zn, Rb, Sr, and Ba were detected in the 23 analyzed samples at different concentration. This may be associated with the different cultivation conditions of each sample. However, it should be noted that the most abundant detected elements were phosphorus and potassium, as we expected since N, P, and K are commonly added in fertilizers formulations. Furthermore, there is a close relationship between the concentration of these elements and the content of tea-quality compounds (mainly polyphenols) (Lin et al., 2012; Mudau et al., 2007; Wang et al., 2020). In addition, other micronutrients which are important nutrients such as Mg, B, Cu, Fe, Mn, Zn, Ca, and Ni, were also detected in lower concentrations.

Moreover, the concentration of heavy metals (Pb, As, Hg, and Cd) were lower than the detection limit for these metals (LOD 3 μg/L) in the 23 analyzed samples. LOD was estimated using the 3s approach from the standard deviation of 5 blank sample measurements. Heavy metals are a group of metals and metalloids that have relatively high density and are toxic even at trace levels. For this reason, their concentrations in food must remain below the limits set by the World Health Organization (WHO). The WHO 2008 permissible limit for Hg, As, and Pb in drinking water was 10 μg/L and 3 μg/L for Cd (Cobbina et al., 2015). Therefore, it can be stated that tea samples and infusions of other plants analyzed in the present work can be safely consumed.

Finally, Minitab software was used to perform a linear discriminant analysis (LDA) using the concentration values of Na, Mg, Al, P, K, Ca, Mn, Fe, Ni, Cu, Zn, Rb, Sr, and Ba. For those cases where the metal concentration was BDL, a value was assigned as the detection limit split by 3 (LOD/3). First, the correlation coefficients (r) between variables were calculated (see Figure S5.A, Supporting Information). The obtained absolute r values were in some cases ± 0.7, indicating a strong linear correlation between variables (Ratner, 2009). Accordingly, the 4 most strongly correlated variables (Sr, Al, P and Mg) were eliminated (see Figure S5.B, Supporting Information). Thus, the LDA was applied to 10 variables (Na, K, Ca, Mn, Fe, Ni, Cu, Zn, Rb, and Ba) (see Table S5, Supporting Information) and 22 of 23 samples were classified into their theoretical groups. Only sample S8 was classified as rooibos when the pre-established group for this sample is black tea. When observing the linear discriminative function (see Table S6, Supporting Information), it can be concluded that Mn (absolute variation of 7) and Rb (absolute evaluation of 5) are the variables with the greatest influence on the classification of the groups. In addition, the group means (see Table S7, Supporting Information) allows to determine that both variables, Mn and Rb, present large values for the black tea and green tea groups and small values for the rooibos and other infusions groups. These results agree with the data published by other authors who also found higher concentrations of Mn and Rb in tea infusions compared to mint infusions (Marguí & Voutchkov, 2018). Thus, misclassification of S8 can be assigned to unexpected small values of Mn and Rb. Nonetheless, Mn and Rb concentrations were the key factors for classifying different herbal infusions, with high values representative of tea leaf infusions and low values indicative of other herb leaf infusions.

It is worth mentioning that tea flavonoids contain catechol and/or galloyl moieties, such as epigallocatechin gallate or brevifolincarboxilic acid, can form complexes with various metal ions (Herman et al., 2022). Thus, higher concentration of Mn and Rb in green and black tea samples compared to other herbal infusions could be strongly correlated with the presence of these specific polyphenols. Therefore, from the combination of the polyphenol and the mineral profiles of the different analyzed infusion samples, a mixed marker can be selected for the samples' classification.

To confirm this affirmation the data from both, polyphenolic content (gallocatechin, gallocatechin gallate, brevifolincarboxilic acid, ellagic acid, astilbin, piscidic acid, and 2-(E)-feruloyl-D-galactaric acid) and mineral composition (Na, K, Ca, Mn, Fe, Ni, Cu, Zn, Rb, and Ba), was combined to perform a statistical analysis. Given that gallocatechin and gallocatechinin gallate are highly correlated (r = 0.98), and astilbin and piscidic acid are also highly correlated (r = 0.95), only gallocatechin, brevifolincarboxylic acid, ellagic acid, piscidic acid, 2-(E)-feruloyl-D-galactaric acid, Na, K, Ca, Mn, Fe, Ni, Cu, Zn, Rb, and Ba are included in the discriminant model. The LDA successfully classified all 23 samples into their respective groups based on their mineral and organic profiles. Observing the linear discriminative function showed that gallocatechin (absolute variation of 43) and manganese (absolute variation of 26) had the most significant influence on the classification. Furthermore, comparing the summary of classified samples from the linear discriminant analysis considering both the mineral and the organic variables, the probability in the classification reaches outstanding values in front of the classification considering only the mineral composition (see Table S8 in the Supporting Information). The findings supported the importance of using combined markers, including specific organic compounds and minerals, such as Mn and Rb, concentrations, for accurately identifying herbal infusion samples. By considering both the organic compound profiles and manganese/rubidium levels, a more reliable classification of the samples was achieved. This approach highlights the importance of integrating multiple parameters to ensure accurate and robust identification of various herbal infusion samples.

In this way, the selected characteristic markers are more reliable. The selected markers are the following for each group of samples:

- Green tea: high content of epigallocatechin/gallocatechin and epigallocatechin gallate/gallocatechin gallate, and high levels of Mn and Rb.
- Black tea: high content of brevifolincarboxylic acid and ellagic acid, and high levels of Mn and Rb.
- Rooibos: high content of astilbin/smitilbin and piscidic acid, and low levels of Mn and Rb.
- Other herbal infusions: high content of 2-(E)-feruloyl-D-galactaric acid, and low levels of Mn and Rb.

These markers can be used in the prevention of food fraud.

5. Conclusions

The 23 commercial samples of tea and other herbal infusions were analyzed by UHPLC-QTOF to determine their polyphenol profile. For this purpose, different workflows have been applied: target, suspect, and unknown analysis. The concentration of 8 polyphenols (benzoic acid, salicylic acid, catechin, rosmarinic acid, cinnamic acid, gallic acid, vanillic acid, and vanillin) was determined using target analysis. From the suspect analysis, 59 polyphenolic compounds mentioned in the literature were identified at different levels of confidence. Moreover, using an unknown analysis workflow, 29 extra polyphenols were putatively found.

Using a semi-quantitative ICP-MS analytical method, an inorganic compound profile of 23 samples of tea and other herbal infusions has been characterized. A screening of several elements of the periodic table was possible for every single injection using a semi-quantitative software package. This confirmed that the concentration of heavy metals (Pb, Cd, Hg, and As) did not exceed the permissible limits recommended by the WHO. Therefore, the analyzed samples can be safely consumed.

In addition, MarkerView statistical software has been used to analyze the UHPLC-QTOF raw data. Thus, characteristic compounds have been found that allow samples to be grouped according to the type of herbal infusion. Samples were classified into 4 groups: green tea, black tea, rooibos, and other herbs. For each group, at least one characteristic polyphenolic compound was potentially identified. Moreover, Minitab was used to perform a linear discriminant analysis of the mineral composition results. Thus, it was determined that a high content of Mn and Rb is indicative of green and black teas, whereas low content of Mn and Rb is representative of rooibos and other herbal infusions. Moreover, the combination of the information provided by polyphenolic and mineral profiles in each sample can be used to classify these samples and prevent food fraud. Characteristics markers have been established to group the infusions. A high content of (epi)gallocatechin and (epi)gallocatechin gallate and high levels of Mn and Rb are indicative of green tea; high content of brevifolincarboxylic acid and ellagic acid, and high levels of Mn and Rb are indicative of black tea; high content of astilbin/smitilbin and piscidic acid, and low levels of Mn and Rb are indicative of rooibos; high content of 2-(E)-feruloyl-D-galactaric acid, and low levels of Mn and Rb are indicative of other herbal infusions.

Associated content

Abbreviations

BA, benzoic acid; C, catechin; CA, cinnamic acid; CE, collision energy; DA, discriminant analysis; DP, declustering potential; ESI, electrospray ionization; GA, gallic acid; ICP-MS, inductively coupled plasma mass spectrometry; LC-QTOF, liquid chromatography coupled triple quadrupole time-of-flight; LDA, linear discriminant analysis; LOD, limit of detection; LOQ, limit of quantification; MSI, metabolomics standards initiative; PCA, principal component analysis; RA, rosmarinic acid; RT, retention time; SA, salicylic acid; S/N, signal-to-noise-ratio; V, vanillin; VA, vanillic acid, WHO, World Health Organization.

Compliance with Ethical Standards

Author Contributions

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Conflict of Interest

Authors declare that they has no conflict of interest.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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