

Determination and comparison of metal contents in simulated body fluid medium conditions of the plant species by flame atomic absorption spectrometry (FAAS)

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ABSTRACT: The importance of plants in folk medicine and scientific studies is increasing day by day. In this context, the plants pose a danger to human health when they are eaten by the public, consumed as herbal tea or used as medicinal plants especially the toxic metals in their composition. For this reason, determining the macro, micro and toxic element content in the plants is important for health. In this study; the metal contents of root and aerial parts of nine different *Salvia* species were determined and the chemometric evaluation of the obtained results was made. In the principal component analysis (PCA) made with 20 elements of *Salvia* species, it was determined that the first two principal components explained 62.60% of the variance and the first six principal components explained 92.70%. When PCA and hierarchical cluster analysis (HCA) results are evaluated; the parts of *Salvia* species root and aerial parts were determined that were not clearly separated and there was no regional grouping. In addition, *Salvia* species prepared as herbal tea was left to different simulated body fluid medium conditions, and the changes in metal contents were examined. It was determined that were taken up Cr, Cd, Pb, Cu, Zn metals in the simulated saliva fluid (SSF), Ni, Mn metals in the simulated gastric fluid (SGF), and Na, K metals in the simulated intestinal fluid (SIF). It was determined that Fe metal in almost all *Salvia* species was not taken from three simulated body fluid mediums. Thus, elements determined which were taken up in body fluid mediums.

KEYWORDS: *Salvia*; chemometry; SSF; SGF; SIF; FAAS.

1. INTRODUCTION

The Lamiaceae family is represented by more than 245 genera and 7886 species worldwide [1]. This family is represented by 45 genera, 558 species, and 742 taxa in the flora of Turkey [2].

The genus *Salvia* L. belongs to the Lamiaceae family and is represented by more than 1000 species worldwide [3-8]. This genus is represented by 53 endemic and 101 species in the flora of Turkey [9]. Species of this genus and the essential oils obtained from them are used in herbal tea, culinary herb, food preservative, food flavoring, cosmetics, perfumery, and the pharmaceutical industry [4,7,9-13].

WHO (World Health Organization) states that the maximum allowable concentration levels in raw plant materials for cadmium, arsenic, and lead are 0.3, 1.0, and 10 mg/kg, respectively [14].

Plants easily absorb macro, micro, and toxic elements from the soil through their roots. Thus, the use of plants by humans may pose health risks. Therefore, it is important to determine the macro, micro, and toxic element contents in the plant. These metal contents are determined used various methods such as Atomic Absorption Spectrometry (AAS), Flame Atomic Absorption Spectrometry (FAAS), Graphite Furnace Atomic Absorption Spectrometry (GF-AAS), Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Inductively Coupled Plasma Mass

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Spectrometry (ICP-MS), Instrumental Neutron Activation Analysis (INAA), and X-ray Fluorescence Spectrometry (XRF) [15-21].

In this study; Metal contents of root and aerial parts of nine different *Salvia* species were determined. The chemometric evaluation of the metal content results obtained was performed. In addition, these species were prepared as herbal tea and left to three different simulated body fluid medium conditions and the changes in metal contents were examined. Thus, the initial metal concentrations in the species were compared with the metal concentrations in simulated body fluid medium conditions.

2. RESULTS AND DISCUSSION

2.1. Metal concentrations of dried the species

When the metal analysis results of *Salvia* species are evaluated; it was determined that the Na, Mg, Fe, Al, Ti, Cu, Cr, Mn, Ni, and Co metal contents of SC7 species were higher than the other species. High metal concentrations of this species were determined as 3805, 17638, 21803, 19152, 3070, 46.38, 71.61, 492, 159, and 13.33 mg/kg, respectively. The Be and Se metal contents of SC8 species were determined that be higher than the other species and metal concentrations were determined as 0.54 and 14.00 mg/kg, respectively. The Zn and Pb metal contents of SC11 species were determined that be higher than the other species and metal concentrations were determined as 145 and 33.45 mg/kg, respectively. Li and Ba metal contents of the SC17 species were determined to be higher than other species and metal concentrations were determined as 103 and 139 mg/kg, respectively. Cd metal content of SC12 species was determined to be higher than other species and Cd metal concentration was determined as 6.72 mg/kg. B metal content of SC15 species was determined to be higher than other species and the metal concentration was determined as 210 mg/kg. K and Mo metal contents of SC1 species were determined that they be higher than the other species and the metal concentrations were determined as 55623 and 18.34 mg/kg, respectively. In addition, Be metal content of SC4, SC12, SC16 species and Se metal contents of SC2, SC3, SC14, SC15, SC18, SC19 species could not be detected (Table 4). The metal content of the samples belonging to different species of the same genus be different; it can be said that it changes depending on genetic factors, geographical location, climatic factors, vegetation period, air pollution, and environmental factors.

Table 4. Metal analysis results of *Salvia* species (mean concentration \pm standard deviation (mg/kg), n = 3)

	SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8	SC9	SC10
Na	2466 \pm 59	2224 \pm 23	2226 \pm 9	2592 \pm 20	2381 \pm 12	3498 \pm 20	3805 \pm 15	3475 \pm 33	2669 \pm 33	3209 \pm 25
K	55623 \pm 103	12958 \pm 62	27609 \pm 231	11760 \pm 177	12914 \pm 9	19458 \pm 80	17780 \pm 77	4605 \pm 39	16073 \pm 114	17201 \pm 34
Mg	6750 \pm 152	1897 \pm 6	4406 \pm 44	1809 \pm 28	5583 \pm 8	5928 \pm 25	17638 \pm 32	10656 \pm 137	7163 \pm 39	5403 \pm 37
Fe	2265 \pm 35	1212 \pm 3	2750 \pm 24	232 \pm 3	931 \pm 7	4004 \pm 23	21803 \pm 192	16686 \pm 127	2850 \pm 16	5892 \pm 76
Al	3266 \pm 63	1691 \pm 6	3455 \pm 28	306 \pm 1	1472 \pm 12	5000 \pm 34	19152 \pm 107	14593 \pm 125	3530 \pm 6	8735 \pm 24
B	151 \pm 4	179 \pm 3	207 \pm 10	136 \pm 2	162 \pm 4	148 \pm 1	182 \pm 1	113 \pm 2	183 \pm 1	195 \pm 3
Ti	233 \pm 2	70.25 \pm 1.58	150 \pm 2	20.96 \pm 0.59	67.65 \pm 0.44	400 \pm 5	3070 \pm 14	317 \pm 4	165 \pm 1	208 \pm 1
Mo	18.34 \pm 0.10	0.87 \pm 0.02	1.37 \pm 0.04	0.35 \pm 0.03	0.47 \pm 0.05	2.09 \pm 0.08	7.14 \pm 0.33	1.02 \pm 0.03	3.46 \pm 0.07	1.42 \pm 0.18
Li	0.66 \pm 0.07	0.65 \pm 0.05	2.70 \pm 0.15	N.D.	0.46 \pm 0.56	1.78 \pm 0.02	8.91 \pm 0.78	12.15 \pm 0.64	2.60 \pm 0.02	7.74 \pm 0.72
Cu	23.56 \pm 0.50	6.88 \pm 0.04	7.53 \pm 0.06	7.82 \pm 0.02	9.59 \pm 0.02	27.03 \pm 0.16	46.38 \pm 0.33	20.28 \pm 0.01	17.03 \pm 0.06	34.12 \pm 0.44
Be	0.01 \pm 0.00	0.06 \pm 0.00	0.12 \pm 0.01	N.D.	0.01 \pm 0.00	0.08 \pm 0.00	0.47 \pm 0.01	0.54 \pm 0.02	0.08 \pm 0.00	0.29 \pm 0.01
Cr	4.26 \pm 0.07	4.88 \pm 0.02	8.65 \pm 0.03	0.99 \pm 0.02	2.86 \pm 0.01	14.57 \pm 0.04	71.61 \pm 0.48	17.52 \pm 0.12	6.73 \pm 0.04	12.80 \pm 0.04
Mn	134 \pm 2	75.16 \pm 0.17	103 \pm 1	37.31 \pm 0.20	45.71 \pm 0.23	143 \pm 1	492 \pm 2	386 \pm 5	144 \pm 1	197 \pm 1
Ni	14.21 \pm 0.21	8.97 \pm 0.01	25.68 \pm 0.14	2.29 \pm 0.01	6.98 \pm 0.04	29.99 \pm 0.06	159 \pm 1	82.96 \pm 0.59	17.25 \pm 0.02	38.93 \pm 0.45
Co	2.00 \pm 0.05	0.87 \pm 0.02	1.46 \pm 0.03	0.24 \pm 0.01	0.53 \pm 0.02	2.62 \pm 0.06	13.33 \pm 0.08	4.88 \pm 0.08	1.35 \pm 0.02	2.36 \pm 0.00
Zn	62.07 \pm 1.12	33.31 \pm 0.15	46.37 \pm 0.40	29.17 \pm 0.12	37.99 \pm 0.22	68.14 \pm 0.34	124 \pm 1	48.79 \pm 0.04	75.42 \pm 0.37	84.02 \pm 0.61
Se	0.47 \pm 0.09	N.D.	N.D.	1.44 \pm 0.05	5.37 \pm 0.65	2.43 \pm 0.34	2.49 \pm 0.23	14.00 \pm 0.93	0.96 \pm 0.05	2.13 \pm 0.26
Cd	1.27 \pm 0.01	3.51 \pm 0.05	2.18 \pm 0.01	3.75 \pm 0.02	1.58 \pm 0.01	4.22 \pm 0.03	3.59 \pm 0.06	3.25 \pm 0.02	2.91 \pm 0.01	4.31 \pm 0.02
Ba	49.32 \pm 0.17	33.22 \pm 0.46	33.23 \pm 0.32	63.51 \pm 0.52	49.15 \pm 0.18	69.75 \pm 1.01	135 \pm 2	52.68 \pm 0.12	82.92 \pm 1.37	110 \pm 1
Pb	1.67 \pm 0.01	1.05 \pm 0.04	1.89 \pm 0.01	0.59 \pm 0.01	2.94 \pm 0.03	1.19 \pm 0.01	4.69 \pm 0.10	16.08 \pm 0.19	8.62 \pm 0.11	13.00 \pm 0.11

N.D.: No Detected; < LOD

Table 4. continued

	SC11	SC12	SC13	SC14	SC15	SC16	SC17	SC18	SC19
Na	3257±53	3421±22	2770±70	2166±5	2056±14	2875±14	2947±6	2055±22	2219±9
K	55223±825	15298±5	28753±538	20926±115	33783±250	40449±182	42948±323	3790±30	15667±147
Mg	5716±91	7611±77	7756±114	3645±9	11881±86	3634±20	12812±22	1460±8	1925±10
Fe	2312±27	389±1	1660±42	1787±15	2400±20	642±5	5188±49	531±4	187±1
Al	3116±41	515±1	2267±49	1919±20	2609±14	751±3	5873±32	736±4	202±1
B	169±3	163±9	156±1	185±6	210±5	152±4	170±5	154±5	164±5
Ti	159±1	39.95±0.55	183±1	42.12±0.69	69.96±1.44	65.46±0.83	559±5	35.79±0.41	8.95±0.17
Mo	1.63±0.07	6.36±0.28	11.06±0.31	0.80±0.09	1.53±0.03	0.82±0.02	1.55±0.05	0.96±0.05	1.12±0.03
Li	4.67±0.41	28.75±1.29	15.37±1.61	3.67±0.35	34.14±0.62	16.43±0.40	103±1	0.46±0.02	0.53±0.08
Cu	18.06±0.22	17.64±0.16	13.64±0.23	16.09±0.05	16.27±0.18	23.64±0.14	20.81±0.22	8.64±0.07	10.11±0.13
Be	0.06±0.01	N.D.	0.03±0.00	0.06±0.00	0.10±0.00	N.D.	0.14±0.01	0.05±0.00	0.06±0.01
Cr	6.75±0.08	2.15±0.01	6.95±0.15	13.45±0.00	17.83±0.08	2.96±0.01	29.06±0.10	3.38±0.00	1.57±0.01
Mn	100±2	23.45±0.18	58.28±1.53	79.79±0.79	101±1	41.87±0.16	176±1	24.47±0.06	27.58±0.06
Ni	14.76±0.24	4.28±0.03	47.58±0.65	26.97±0.02	33.82±0.10	6.19±0.06	54.03±0.68	9.16±0.06	3.03±0.01
Co	1.05±0.02	0.56±0.02	1.24±0.03	1.73±0.01	2.12±0.02	0.53±0.02	3.51±0.03	0.75±0.02	0.33±0.01
Zn	145±2	95.81±0.23	85.77±1.68	30.74±0.13	52.16±0.32	57.12±0.12	53.97±0.09	23.44±0.23	46.22±0.16
Se	3.61±0.10	1.43±0.34	2.68±0.43	N.D.	N.D.	2.77±0.23	2.17±0.32	N.D.	N.D.
Cd	3.17±0.01	6.72±0.09	5.19±0.02	3.99±0.04	3.55±0.00	1.61±0.01	3.04±0.11	3.75±0.03	1.49±0.02
Ba	120±1	101±1	92.36±0.45	79.78±0.40	98.47±0.33	120±1	139±6	14.84±0.10	5.60±0.06
Pb	35.43±0.30	2.00±0.06	1.34±0.01	0.79±0.01	1.68±0.01	0.48±0.01	1.60±0.09	0.55±0.01	1.79±0.02

N.D.: Not Detected; < LOD

When the metal contents of the root and aerial parts of the same species are compared; it was determined that most of the metal contents of the aerial parts were higher than the root. Table 4. It was determined that the majority of the species found were as above.

Pb metal contents of only SC8, SC10, and SC11 samples of *Salvia* species were found to be higher than the acceptable maximum concentration values in raw plant materials of WHO. In addition, Cd metal contents of all species were found to be higher than the maximum acceptable concentration values in raw plant materials of WHO. If the species is to be used for herbal tea or medicinal purposes, the toxic dose relationship of Cd and Pb metal contents should be considered.

2.2. Chemometric evaluation of the species

The root and aerial parts of nine different *Salvia* species 20 elements (Na, K, Mg, Fe, Al, B, Ti, Mo, Li, Cu, Be, Cr, Mn, Ni, Co, Zn, Se, Cd, Ba, Pb) analysis were performed and chemometric analysis techniques such as PCA and HCA were applied to the obtained data.

2.2.1. Principle component analysis (PCA)

The analysis results of 20 elements for the root and aerial parts of nine different *Salvia* species and formed the data set of the study and the samples were classified against the variables by reducing the number of components with PCA. In the PCA that was done with 20 elements of *Salvia* species, six principal components with eigenvalues greater than one were determined. The first two principal components were determined that explained 62.60% of the variance and the first six principal components explained 92.70% (Table 5). For the first principal component, Fe, Co, Ni, Al, Be, Cu, Mn, Ti, Mg variables were dominant variables and the scores given in Table 6. SC7, SC8, SC10, and SC17 samples were determined to have higher concentrations for these variables. The concentration of elements such as Be and Se according to the 2nd principal component was determined that be higher than the other variables and the samples with the highest concentration for these variables were SC2, SC4, SC5, SC8, SC18, and SC19 samples (shown in bold in Table 5).

Table 5. The loading, eigenvalue, variance and cumulative variance values of the principle components of the species

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Na	0.239	-0.046	-0.341	0.132	0.160	0.146	-0.315
K	0.004	-0.478	-0.135	-0.348	-0.316	-0.012	0.015
Mg	0.272	-0.148	0.081	0.080	-0.201	0.123	0.299
Fe	0.302	0.179	0.027	-0.050	-0.032	-0.020	0.067
Al	0.304	0.147	0.010	-0.049	-0.020	-0.057	0.076
B	0.000	-0.288	0.365	0.093	0.106	-0.547	0.310
Ti	0.278	-0.029	0.220	-0.106	0.142	0.011	-0.229
Mo	0.059	-0.231	0.013	-0.457	0.195	0.543	0.418
Li	0.071	-0.265	0.062	0.411	-0.568	0.194	0.089
Cu	0.268	-0.144	-0.008	-0.080	0.131	0.032	-0.386
Be	0.270	0.259	-0.023	0.040	-0.077	-0.135	0.198
Cr	0.289	-0.045	0.251	0.034	-0.017	-0.052	-0.098
Mn	0.302	0.137	0.025	-0.092	-0.059	-0.046	0.091
Ni	0.303	0.059	0.139	0.004	-0.009	0.025	0.117
Co	0.301	0.038	0.187	-0.075	0.048	0.007	-0.062
Zn	0.183	-0.323	-0.302	-0.082	0.296	-0.141	0.052
Se	0.139	0.323	-0.408	0.028	-0.289	0.100	0.130
Cd	0.050	-0.085	-0.106	0.586	0.480	0.225	0.327
Ba	0.181	-0.393	-0.134	0.260	-0.101	-0.024	-0.260
Pb	0.090	-0.052	-0.517	-0.105	0.003	-0.470	0.228
Eigenvalue	9.862	2.664	2.082	1.436	1.271	1.229	0.547
Variance (%)	49.30	13.30	10.40	7.20	6.40	6.10	2.70
Cumulative (%)	49.90	62.60	73.00	80.20	86.60	92.70	95.50

Larger number indicate a significant contribution to the separation along the principal component (PC) axes. The highlighted bold numbers are the major contributors to each principal component

Table 6. The score values of the detected metals in the species

Samples	PC1	PC2	PC3	PC4	PC5	PC6	PC7
SC1	-0.744	-1.470	0.132	-3.652	-0.281	1.928	0.488
SC2	-2.441	1.011	1.039	0.232	0.500	-0.498	0.269
SC3	-1.555	0.271	1.518	-0.636	-0.207	-1.417	0.708
SC4	-2.616	1.226	-0.220	0.594	0.333	0.706	-0.781
SC5	-2.069	1.365	-0.047	-0.413	-0.804	-0.233	-0.293
SC6	0.629	0.366	-0.541	0.381	0.967	0.780	-1.110
SC7	10.341	-0.124	2.160	-0.732	1.217	-0.163	-0.550
SC8	4.531	4.458	-2.453	0.137	-1.461	0.520	0.878
SC9	-0.449	-0.252	-0.039	-0.152	0.457	-0.741	0.233
SC10	1.821	-0.278	-0.670	0.758	0.989	-1.381	-0.099
SC11	0.449	-2.342	-3.773	-0.854	0.148	-2.176	0.141
SC12	-0.728	-1.470	-1.171	2.083	1.625	1.560	0.380
SC13	-0.328	-1.161	-0.541	0.185	0.849	1.798	1.062
SC14	-1.454	0.050	1.233	0.594	0.328	-0.514	-0.024
SC15	-0.285	-1.542	1.650	0.820	-0.978	-0.832	1.015
SC16	-1.325	-0.921	-0.692	-0.419	-1.116	0.201	-1.877
SC17	2.062	-2.116	0.745	1.699	-3.142	0.748	-0.160
SC18	-2.919	1.749	0.940	0.340	0.653	0.207	0.040
SC19	-2.920	1.181	0.731	-0.963	-0.079	-0.495	-0.321

Bold numbers indicate the samples showing the highest effect given in Table 6.

When Figure 2 and Figure 3 were evaluated together, it was determined that there was no complete separation. The root and aerial parts of *Salvia* species was determined that the parts of it were not clearly separated and there was no regional grouping. However, looking at the variables, it can be said that some

samples are more similar to each other and that there may be grouping according to the variables. Accordingly, SC2, SC4, SC5, SC8, SC18, and SC19 coded samples are located in the upper left part of the score graph and it was determined that a group was formed. The samples in this group were collected from Van and Elazig provinces, and it was determined that they had the lowest concentration in terms of metal content. At the same time, the SC2, SC4, SC8, SC18 coded examples are aerial and the others are root belonging to the samples.

A second group was determined to consist of SC3, SC1, SC9, SC12, SC13, SC14, SC16 coded samples. As in the 1st group, samples belonging to both the root and aerial parts are members of this group. K, B, and Mo elements were found to be higher in the samples in this group than in the other samples. Another group was determined to consist of SC6, SC10, SC11, and SC17 samples. There is no regional similarity in the samples belonging to this group. However, it was determined that the amounts of elements such as Mo, Li, Ba, Zn, Pb, Cd were similar in these samples, which was effective in separating these samples from the others.

The ratio of Se, Be, Al, Fe, and Mn elements in the SC8 sample belonging to the part aerial of *Salvia pseudoeuphratica* species was considerably higher than the other samples and it was determined that they were in the upper right part of the score graph alone. the SC7 coded sample belonging to the part root of *Salvia pinnata* species was determined that differed from the other samples in terms of the concentration of Co, Na, Ti, Cr, Cu, Mg, Ni, Al, Fe, Mn, Zn, Ba, Cd elements. The high metal concentration in these two samples was determined that both of them were grouped separately and completely separated from all other samples (Figure 4).

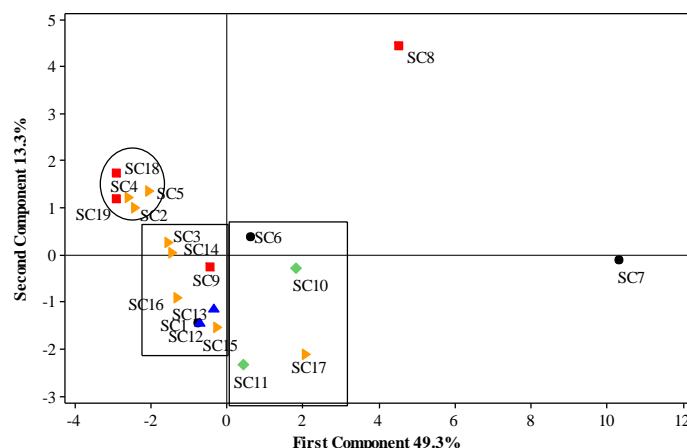


Figure 2. Score plot of the first two principal components (PC1 and PC2) of *Salvia* species.

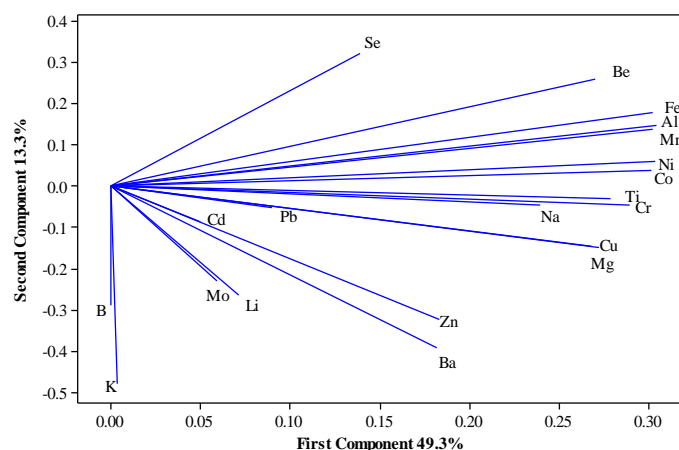


Figure 3. Loading plot of the first two principal components (PC1, PC2) of *Salvia* species.

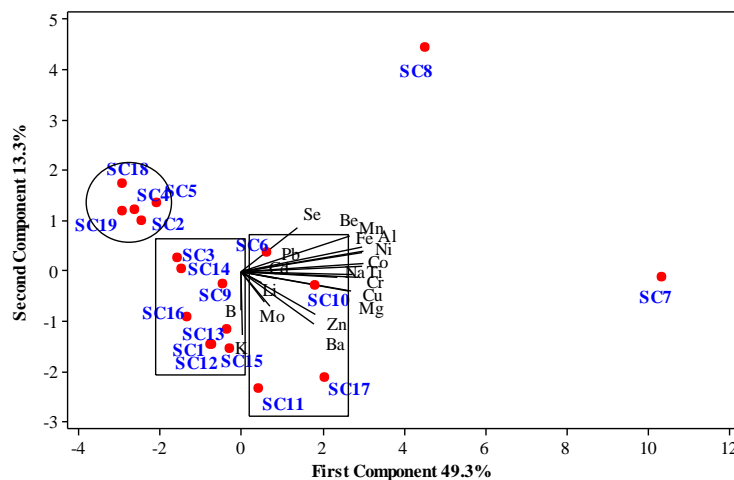


Figure 4. Biplot plot of the first two principal components (PC1, PC2) of *Salvia* species.

2.2.2. Hierarchical cluster analysis (HCA)

According to the dendrogram related the HCA' prepared with the results of metal analysis of *Salvia* species have been consisting of five clusters and these clusters are; Cluster 1: SC1, SC3, SC14, SC9, SC15, SC16; Cluster 2: SC6, SC10, SC12, SC13, SC11, SC17; Cluster 3: SC2, SC18, SC19, SC4, SC5; Cluster 4: SC7 and Cluster 5: SC8 was determined to consist of samples (Figure 5). In the HCA analysis, samples in the root and aerial parts were determined that were not segregated. In addition, it was determined that regional differences did not affect the formation of groups (Similarity=49.75). *Salvia* species that were analyzed Fe, Co, Ni, Al, Be, Cu, Mn, Ti, Mg elements were and were found to be the main elements of fingerprints.

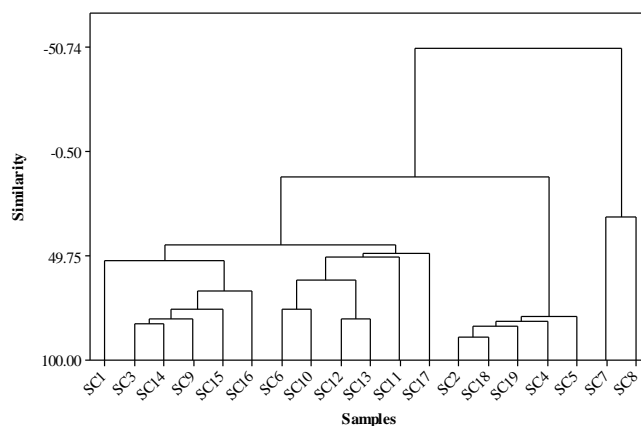


Figure 5. Dendrogram of *Salvia* species prepared according to observations (Euclidean distance and Ward Linkage method).

2.3. Metal analysis results in simulated body fluid mediums of the species

2.3.1. Metal analysis results in simulated saliva fluid medium (SSF)

When the metal contents of the species in SSF medium conditions are compared; it was determined that the Fe, Cu, Cr, Mn, Ni, and Zn metal contents of SC7 species were higher than the other species. The Na and K metal contents of SC17 species were determined that be higher than the other species. In addition, the Pb

metal content of SC11 species was determined that and Cd metal content of SC13 species were higher than other species (Table 7).

Table 7. Metal analysis results of simulated saliva fluid medium of *Salvia* species (mg/kg)

Elements	SC3	SC5	SC7	SC9	SC11	SC13	SC15	SC17	SC19
Na	180±11	216±11	237±29	233±9	222±7	221±12	201±19	291±27	272±17
K	1053±42	324±11	514±17	635±45	1805±48	1099±38	2670±490	5438±261	1941±145
Fe	154±3	150±15	175±14	146±9	141±6	154±12	158±9	129±7	137±10
Cu	3.07±0.22	4.75±0.15	12.93±1.49	7.77±0.36	7.95±0.58	6.16±0.44	7.71±0.29	9.57±0.40	4.82±0.41
Cr	6.84±0.37	2.60±0.10	56.77±3.65	5.02±0.23	5.05±0.32	4.86±0.18	15.46±1.07	25.27±0.77	1.38±0.04
Mn	44.21±3.75	17.02±1.18	125±5	56.37±2.29	38.68±1.68	16.73±0.51	40.57±2.54	61.91±2.85	8.75±0.16
Ni	9.48±0.00	N.D.	33.81±0.84	2.29±0.06	N.D.	N.D.	9.37±0.22	16.20±1.03	0.87±0.02
Zn	22.92±0.29	19.15±1.07	49.36±3.23	25.30±0.77	74.16±3.86	30.40±0.30	27.91±0.43	32.44±1.22	37.11±0.30
Cd	1.49±0.19	1.21±0.15	2.51±0.14	1.99±0.14	2.52±0.35	3.61±0.29	2.24±0.32	1.45±0.29	0.93±0.02
Pb	0.65±0.18	2.73±0.08	4.26±0.32	7.83±0.81	33.17±0.43	0.94±0.09	0.83±0.16	0.64±0.08	0.54±0.03

N.D.: No Detected : < LOD

When the species in Tables 4 and 7 are evaluated together; Cr, Cd, Pb metal contents of SC5, SC7, SC9, SC11, and SC13 species, Zn Fe, Cd, Cr metal contents of SC19 species, Cr, Cd metal contents of SC3 and SC15 species, Zn, Cr metal contents of SC17 are largely was determined to remain in the SSF. The metal contents of Cr in nine species, Cd in eight species, Pb in five species, Zn in two species, and Fe in one species of *Salvia* were determined that remained in the SSF (Table 7). Cr, Cd, Pb metal in plants and foods can be said that be mostly taken in SSF and passed into the living body in this way.

Cu metal content of SC19, SC9, SC13 species, Zn metal content of SC3 species, Cu, Cd metal contents of SC17 species, Cu, Zn metal contents of SC5, SC11 species, and Cu, Zn, Pb metal contents of SC15 species approximately 50% and more were determined to remain in SSF. Cu in seven species, Zn in four species, Cd and Pb in one species of *Salvia* genus approximately 50% or more was determined that remained in SSF (Table 7). Approximately 50% and more of Cu metal in plants and foods can be said that is taken in SSF and passed into the living body in this way.

Na, K, Mn, Ni, Pb of SC19; Na, K, Fe, Mn, Ni of SC5, SC11, SC15; Na, K, Fe, Mn, Ni, Zn of SC9, SC13; Na, K, Fe, Mn, Ni, Pb of SC17; Na, K, Fe, Cu, Mn, Ni, Pb of SC3 and Na, K, Fe, Cu, Mn, Ni, Zn metal contents of SC7 species was determined that remained a small amount in SSF. The metal contents of Na, K, Mn, and Ni in nine species, Fe in eight species, Pb and Zn in three species, and Cu in two species of the *Salvia* genus were determined that remained small amounts in SSF (Table 7).

2.3.2. Metal analysis results in simulated gastric fluid medium (SGF)

When the metal contents of the species in SGF medium conditions are compared; the Cu, Cr, Mn and Ni metal contents of SC7 species were determined that be higher than the other species. Na and Fe metal contents of SC3 species was determined that be higher than the other species. Zn and Pb metal contents of SC11 species were determined that be higher than the other species. In addition, the K metal content of SC17 species was determined to be higher than other species (Table 8).

Table 8. Metal analysis results of simulated gastric fluid medium of *Salvia* species (mg/kg)

Elements	SC3	SC5	SC7	SC9	SC11	SC13	SC15	SC17	SC19
Na	291±15	177±3	140±10	158±13	174±5	273±4	226±13	144±12	190±8
K	780±16	207±4	254±14	335±23	1258±14	690±5	2223±55	2316±130	1126±48
Fe	296±13	219±6	215±12	208±15	252±7	223±9	241±6	197±7	29.11±1.14
Cu	3.64±0.05	4.44±0.12	13.70±0.16	7.23±0.08	6.78±0.11	5.44±0.14	6.98±0.23	9.59±0.06	4.77±0.22
Cr	0.33±0.07	0.10±0.00	2.35±0.12	0.27±0.02	0.29±0.02	0.29±0.03	0.97±0.05	1.81±0.05	0.11±0.01
Mn	52.27±1.99	27.13±0.17	137±5	74.00±2.12	58.2±0.58	37.03±1.64	55.77±2.79	89.65±2.14	16.08±0.15
Ni	15.16±0.23	5.79±0.25	113±3	12.19±0.81	10.54±0.06	45.75±2.42	20.76±1.00	35.85±1.92	2.05±0.11
Zn	28.27±0.21	17.00±0.11	38.57±0.97	18.18±0.35	40.59±0.34	19.99±0.71	18.68±0.11	21.62±1.01	21.64±1.02
Cd	0.63±0.07	0.36±0.03	0.81±0.09	0.40±0.07	0.60±0.07	0.99±0.04	0.74±0.04	0.54±0.08	0.11±0.03

Pb	0.05±0.00	0.14±0.02	0.25±0.07	0.29±0.01	1.41±0.06	0.04±0.00	0.04±0.00	0.03±0.00	0.04±0.00
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When the species in Tables 4 and 8 are evaluated together, Ni metal content of SC5, SC7, SC9, SC11, SC15, SC17, SC19; Zn metal content of SC3, and Mn, Ni metal contents of SC13 determined that mostly remained in SGF. Ni in eight species, Mn and Zn metal contents in one species of *Salvia* genus was determined that remained mostly in the SGF (Table 8). Ni metal in plants and foods can be said that be mostly taken in the SGF and passed into the living body in this way.

Cu, Mn, Zn metal contents of SC5 and SC19; Mn metal content of SC9, SC11, and SC15; Cu, Mn, Ni metal contents of SC3; Cu, Mn metal contents of SC17 species was determined that remained approximately 50% and more in SGF. The metal contents of Mn in seven species, Cu in four species, Zn in two species and Ni in one species of *Salvia* genus were determined that remain approximately 50% or more in SGF (Table 8). Approximately 50% and more of Mn metal in plants and foods can be said that be taken in the SGF and passed into the living body in this way.

Na, K, Fe, Cr, Cd, Pb metal contents of SC3, SC5, SC17, and SC19; Na, K, Fe, Cu, Cr, Mn, Zn, Cd, Pb metal contents of SC7; Na, K, Fe, Cu, Cr, Zn, Cd, Pb metal contents of SC9, SC11, SC13 and SC15 and the Zn metal content of SC17 species determined that remained in small amounts in SGF. Metal contents of Na, K, Fe, Cr, Cd and Pb in nine species, Zn in six species, Cu in five species and Mn in one species of *Salvia* genus was determined that remained in SGF (Table 8). Na, K, Fe, Cr, Cd and Pb metals in plants and foods can be said that are almost not taken into SGF and they pass into the living body in very small amounts.

2.3.3. Metal analysis results in simulated intestinal fluid medium (SIF)

When the metal contents of the species in SIF medium conditions are compared; Cr, Mn and Ni metal contents of SC7 species were determined that be higher than the other species. K, Zn and Pb metal contents of SC11 species were determined that be higher than the other species. In addition, the metal contents of K of SC3, Fe of SC5, Cd of SC13, and Cu of SC17 species were determined that be higher than other species (Table 9).

Table 9. Metal analysis results of simulated intestinal fluid medium of *Salvia* species (mg/kg)

Elements	SC3	SC5	SC7	SC9	SC11	SC13	SC15	SC17	SC19
Na	1986±9	1964±30	1975±32	1921±25	1916±109	1790±243	677±33	693±5	687±16
K	21567±263	7063±142	11031±503	14049±138	44200±829	23617±423	21670±537	26780±743	12202±258
Fe	22.48±0.74	25.56±0.65	15.87±0.22	13.60±0.86	14.18±0.71	14.12±0.59	16.41±1.10	16.96±0.90	16.25±0.80
Cu	0.13±0.01	0.19±0.01	0.60±0.01	0.28±0.01	0.39±0.02	0.36±0.02	0.48±0.01	0.72±0.01	0.38±0.01
Cr	0.33±0.02	0.12±0.01	2.72±0.07	0.31±0.01	0.32±0.01	0.31±0.01	0.91±0.02	0.93±0.06	0.05±0.00
Mn	4.86±0.16	5.22±0.29	44.96±0.78	12.26±0.48	2.42±0.09	4.13±0.18	4.45±0.17	20.69±0.70	2.27±0.19
Ni	0.54±0.07	0.24±0.03	3.59±0.12	0.28±0.04	0.20±0.06	1.64±0.44	1.27±0.13	1.37±0.17	0.08±0.00
Zn	4.92±0.14	4.11±0.11	13.19±0.32	3.38±0.17	17.41±0.28	10.32±0.31	3.97±0.20	8.23±0.11	8.89±0.21
Cd	0.01±0.00	0.02±0.00	0.04±0.00	0.02±0.00	N.D.	0.06±0.00	0.04±0.00	0.02±0.00	0.02±0.00
Pb	N.D.	0.02±0.00	0.04±0.00	0.05±0.00	0.15±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00

N.D.: No Detected : < LOD

When the species in Tables 4 and 9 are evaluated together; Na, K metal contents of SC3, SC9 and SC13 species, K metal content of SC7, SC11, SC15, SC17 and SC19 species and Na metal content of SC5 species was determined that remained in SIF. The metal contents of the K in eight species and Na in four species of nine *Salvia* genus was determined that remain mostly in SIF (Table 9). The K metal in plants and foods can be said that be mostly taken into SIF and passed into the living body in this way.

The Na metal content of SC7 and SC11 species and the K metal content of SC5 species was determined that remain approximately 50% and more in SIF. Na metal contents in two species and K metal contents in one species of nine *Salvia* genus was determined that remain approximately 50% or more in SIF (Table 9).

Na, Fe, Cu, Cr, Mn, Ni, Zn, Cd and Pb metal contents of SC15, SC17 and SC19 species; Fe, Cu, Cr, Mn, Ni, Zn Cd and Pb metal contents of SC3, SC5, SC7, SC9, SC11 and SC13 species was determined that remained in a very small amount in SIF. The Fe, Cu, Cr, Mn, Ni, Zn, Cd, Pb metal contents in nine species, Na metal contents in three species, of *Salvia* genus was determined that remain in very small amounts in SIF (Table 9). The Fe, Cu, Cr, Mn, Ni, Zn, Cd, Pb metals in plants and foods can be said that are almost not taken into SIF and cannot enter the living body.

When the *Salvia* species in the simulated body fluid mediums in Tables 7, 8 and 9 were evaluated together, the metal contents of Cr, Cd and Pb in all species were determined that remained mostly in the SSF. In addition, Cu metal in seven species and Zn metal in six species was determined that remain 50% and more in SSF. Cr, Cd, Pb, Cu, and Zn metals and their carbonate, oxide, sulfide, and salts in plants and foods are mostly absorbed in saliva fluid. These metals can be said to pass in the saliva medium into human and animal bodies. Ni metal content in eight species of nine *Salvia* genus was determined that remain to be largely in SGF. In addition, approximately 50% and more of Mn metal in seven species were determined that remain in SGF. The Ni and Mn metals are absorbed in the gastric fluid medium and it can be said that it was taken into the living body in this way. The Na and K metal contents of almost all *Salvia* species were determined that remained largely in SIF. As a result, Na and K metals are absorbed in the intestinal fluid medium and it can be said that they are taken into the living body by this way.

The Fe metal in almost all of *Salvia* species was determined that were not taken from three simulated body fluid mediums. Since the soluble Fe metal content in the soil is very low, it can be said that the plants cannot take the Fe metal into their structures. Therefore, Fe metal contents can be said that cannot be taken by plants into body fluid mediums.

3. CONCLUSIONS

There are mineral and toxic elements in plants. These elements both have positive effects on human health and pose a health hazard. Therefore, it is very important to determine the toxic and mineral metal content of *Salvia* species.

In this study, the metal content of *Salvia* species was examined and the chemometric evaluation of the obtained data was made. Na, Mg, Fe metal contents of SC7 species can be said that has higher than other species, and the mineral elements of this species are more beneficial to human health. In addition, Cu, Cr, Mn, Ni, and Co metal contents of this species be higher than other species, it can be said that it should be used carefully considering the negative relationship in terms of health the elements above the threshold concentration levels of the microelement contents of this species.

According to the PCA analysis results made by 20 variables, the root and aerial parts of *Salvia* species, when the loading and score graphs of the first two main components (PC1, PC2) were evaluated together, it was determined that there was no complete separation. In addition, the parts of these species the root and aerial parts have been determined that are not clearly separated and there is no regional grouping. According to the HCA analysis results, the root and aerial parts were determined that not to have occurred separation in the samples of the parts.

The changes in the metal content of *Salvia* species were investigated by leaving three different simulated body fluid mediums. When the results obtained in simulated body fluid medium conditions are evaluated as a whole, five (Cr, Cd, Pb, Cu and Zn) of the ten metals examined in the study can be said that remains in the saliva fluid medium and thus 50% and more of the metals are taken in the mouth. Two each metal in the gastric (Ni, Mn) and intestinal (K, Na) fluid medium was determined that remained. Thus, which metal has been determined that absorbed where in the human body.

4. MATERIALS AND METHODS

4.1. Plant materials

The root and aerial parts of nine different *Salvia* species were collected from various regions of Turkey and dried following the literature. The dried plant samples were identified and stored by Mehmet Firat in the Faculty of Science Herbarium (VANF) of the University of Van Yüzüncü Yıl (Table 1).

Table 1. Information about of studied species

Plant species	Species Codes	Gathering Places	Harvesting Times	Herbarium Number
<i>Salvia multicaulis</i> *	SC1	Diyarbakır	2015	TAGEM/17/ A07/P09/013
<i>Salvia multicaulis</i> Vahl	SC2 (root parts)	Van	2014	M. FIRAT 30656 (VANF)
<i>Salvia multicaulis</i> Vahl	SC3 (aerial parts)	Van	2014	M. FIRAT 30656 (VANF)
<i>Salvia pachystachys</i> Trautv.	SC4 (root parts)	Van	2015	M. FIRAT 30878 (VANF)
<i>Salvia pachystachys</i> Trautv.	SC5 (aerial parts)	Van	2015	M. FIRAT 30878 (VANF)
<i>Salvia pinnata</i> L.	SC6 (root parts)	Diyarbakır	2014	M. FIRAT 31318 (VANF)
<i>Salvia pinnata</i> L.	SC7 (aerial parts)	Diyarbakır	2014	M. FIRAT 31318 (VANF)

<i>Salvia pseudeuphratica</i> Rech.f.	SC8 (root parts)	Elazığ	2015	M. FIRAT 32584 (VANF)
<i>Salvia pseudeuphratica</i> Rech.f.	SC9 (aerial parts)	Elazığ	2015	M. FIRAT 32584 (VANF)
<i>Salvia siirtica</i> Kahraman, Celep & Doğan	SC10 (root parts)	Hakkari	2014	M. FIRAT 30755 (VANF)
<i>Salvia siirtica</i> Kahraman, Celep & Doğan	SC11 (aerial parts)	Hakkari	2014	M. FIRAT 30755 (VANF)
<i>Salvia spinosa</i> L.	SC12 (root parts)	Mardin	2016	M. FIRAT 30908 (VANF)
<i>Salvia spinosa</i> L.	SC13 (aerial parts)	Mardin	2016	M. FIRAT 30908 (VANF)
<i>Salvia suffruticosa</i> Montbret & Aucher ex Benth.	SC14 (root parts)	Van	2014	M. FIRAT 30657 (VANF)
<i>Salvia suffruticosa</i> Montbret & Aucher ex Benth.	SC15 (aerial parts)	Van	2014	M. FIRAT 30657 (VANF)
<i>Salvia xanthocheila</i> Boiss. ex Benth.	SC16 (root parts)	Van	2014	M. FIRAT 30668 (VANF)
<i>Salvia xanthocheila</i> Boiss. ex Benth.	SC17 (aerial parts)	Van	2014	M. FIRAT 30668 (VANF)
<i>Salvia cerino-pruinosa</i> Rech.f. var. <i>cerino-pruinosa</i>	SC18 (root parts)	Elazığ	2015	M. FIRAT 32539 (VANF)
<i>Salvia cerino-pruinosa</i> Rech.f. var. <i>cerino-pruinosa</i>	SC19 (aerial parts)	Elazığ	2015	M. FIRAT 32539 (VANF)

4.2. Chemicals

The following chemicals were purchased from Merck (Germany); NaCl (EMSURE, for analysis), $MgCl_2 \cdot 6H_2O$ (for analysis), CH_3COOK (EMSURE, for analysis), KCl (for analysis), $CaCl_2$ (EMSURE ACS reagent), Lactic acid (EMPROVE), NaBr (EMPROVE), Pepsin (for biochemistry), D(+)-Fructose (for biochemistry), KH_2PO_4 (For EMSURE analysis) and NH_4OH (EMSURE for analysis). In addition, D(+)-Glucose (anhydrous), NaOH (ACS reactive pellet), HNO_3 (70%), H_2O_2 (34.5-36.5%), $CuCl_2 \cdot 2H_2O$ (reactive), Urea (ACS reactive), HCl ($\geq 37\%$ ACS reactive) was purchased from Sigma Aldrich (Germany). While $CaCl_2 \cdot 2H_2O$ (ACS reactive), H_3PO_4 ($\geq 99\%$), NaF (98.5-100.5%) were purchased from Honeywell/Fluka (USA), Uric acid ($\geq 99\%$ for biochemistry) was purchased from Roth (Germany) and $K_3PO_4 \cdot 3H_2O$ (97%) from abcr GmbH (Germany).

4.3. Preparation of plant samples for metal analysis

To analyze the metals of plants belonging to nine different *Salvia* species, previously-dried plant tissues were homogenized and ground in a pot. The tissues were then weighed and placed in microwave teflon tubes, each weighing about 0.1 g. Six mL of HNO_3 and two mL of H_2O_2 were added into each tube in a 6:2 ratio. Using the microwave (MILESTONE ETHOS One), the samples were first microwaved at a 500-w energy level for 15 min and heated up to $300^\circ C$. Following this process, the samples were then heated at a 1500-w energy level for 15 min and held at $300^\circ C$. Finally, the samples were kept at 500 w energy for 10 min while the temperature was reduced gradually from $300^\circ C$ to $90^\circ C$, followed by a 40 min solubilization step in a closed microwave system. After the microwave solubilization procedure, the mixture in Teflon tubes was filtered using blue-banded filter paper. By filling 100 mL volumetric flasks with the obtained supernatant, the mixture was diluted to 100 mL with ultra-distilled water. The diluted samples were then transferred into screw-capped analysis tubes to make them ready for analysis.

4.4. Preparation of simulated body fluid

4.4.1. Preparation of simulated saliva fluid

A total of 1.28 g NaCl was put into 1 L of volumetric flask and dissolved in 500 mL of ultra-distilled water. Then, 0.125 g of $MgCl_2 \cdot 6H_2O$, 0.095 g KCl, 1.508 g CH_3COOK , 0.167 g $CaCl_2$, 0.386 g $K_3PO_4 \cdot 3H_2O$, 0.0042 g NaF, and 0.05 mL H_3PO_4 were added into the solution and mixed. The residue and turbidity in the solution were cleaned by adding one drop of H_3PO_4 . Then, ultra-distilled water was added to the mixture and stirred so that the final volume of the solution was 1 L. The solution was stirred and added lactic acid until reaching the desired pH value. The final pH value of the solution was adjusted to 6.3 [22].

4.4.2. Preparation of simulated gastric fluid

A total of 0.265 g $CaCl_2 \cdot 2H_2O$ was added into a 1 L of volumetric flask and dissolved in 500 mL of ultra-distilled water. Subsequently, 0.153 g $MgCl_2 \cdot 6H_2O$, 0.865 g KCl, 2.856 g NaCl, 0.0008 g NaBr, 0.0009 g NaF, 0.0003 g $CuCl_2 \cdot 2H_2O$, 0.138 g of D(+)-Fructose, 0.350 g of D(+)-Glucose, 0.084 g Urea, 0.0084 g Uric acid, and 3.20 g Pepsin was added to the solution and mixed. Then, the mixture was completed to the final volume of 1 L solution by adding ultra-distilled water. To acquire the desired pH value, 0.04 M HCl and 0.1 M NH_4OH were added to the solution and mixed. The final pH value of the solution was adjusted to 1.54 [23].

4.4.3. Preparation of simulated intestinal fluid

A total of 6.80 g of KH_2PO_4 was put into 1 L of volumetric flask and dissolved in 500 mL ultra-distilled water. Then, 0.90 g of NaOH was added to the solution and mixed. Subsequently, the mixture was completed to the final volume of 1 L solution by adding ultra-distilled water. To acquire the desired pH value, 2 M HCl was added to the solution and mixed. The final pH value of the solution was adjusted to 6.80 [24].

4.5. Preparation of herbal tea

In a 100 mL beaker, 0.5 g *Salvia* leaves were put and rinsed twice with ultra-distilled water to remove dust and residues. On *Salvia* leaves, 50 mL of boiled ultra-distilled water was then added, boiled for 10 min, and left to brew. Finally, the tea in the beaker was filtered with blue-banded filter paper. The resulting filtrate was used in experimental studies.

4.6. Experimental designs of prepared sage tea in simulated body fluid medium

The experiment in three different simulated body fluid medium conditions of prepared herbal tea samples is shown in Figure 1. The procedures indicated in Figure 1 were performed separately for each simulated body fluid medium.

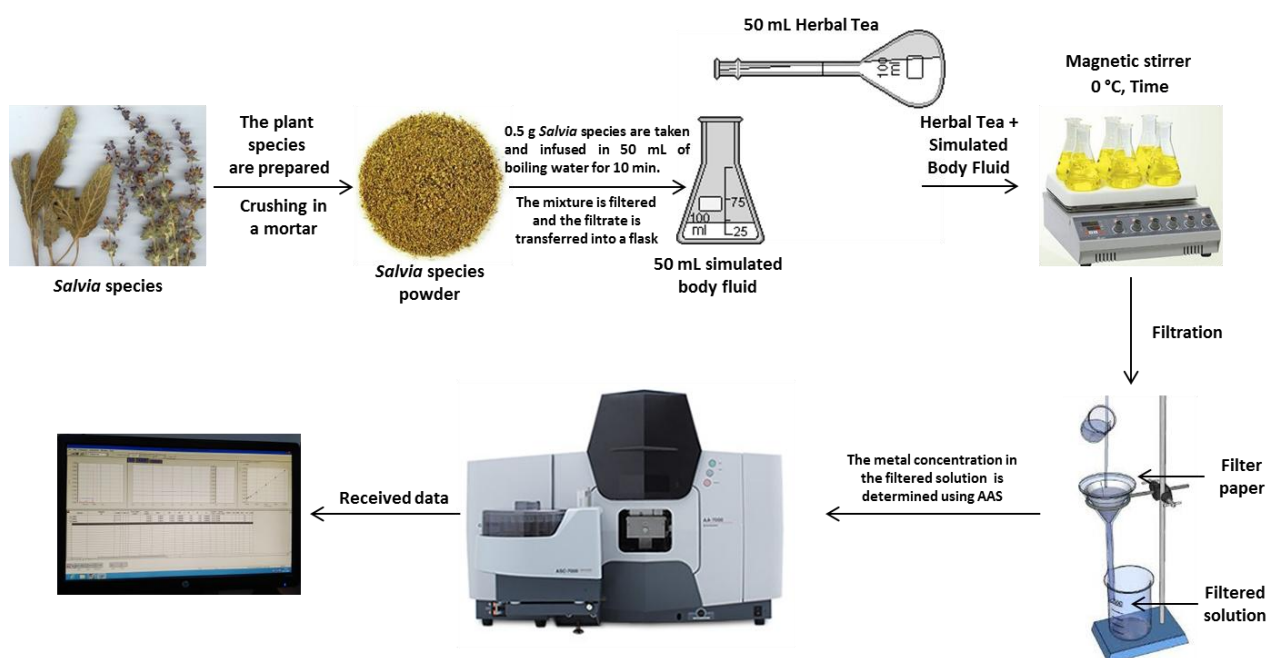


Figure 1. The experiment in different simulated body fluid mediums of *Salvia* species.

4.7. The AAS device and its working conditions

Atomic Absorption Spectroscopy (AA-7000, Shimadzu) was used to assess metal analyses in dry plant samples and prepared herbal tea samples in simulated body fluids (Table 2).

Table 2. AAS instrument operating analytical conditions

Measured Element	Na	K	Fe	Cu	Zn
Wavelength	589 nm	766.5 nm	248.3 nm	324.8 nm	213.9 nm
Slit Width	0.2 nm	0.7 nm	0.2 nm	0.7 nm	0.7 nm
Lamp Current	12 mA	10 mA	12 mA	8 mA	8 mA
Gas Flow Rate	1.8/min.	2 L/min.	2.2 L/min.	1.8 L/min.	2 L/min.
Flame Height	7 mm	7 mm	9 mm	7 mm	7 mm
Flame Type	Air-Acetylene	Air-Acetylene	Air-Acetylene	Air-Acetylene	Air-Acetylene
Measured Element	Ni	Mn	Cr	Cd	Pb
Wavelength	232 nm	279.5 nm	357.9 nm	228.8 nm	283.3 nm
Slit Width	0.2 nm	0.2 nm	0.7 nm	0.7 nm	0.7 nm

Lamp Current	12 mA	10 mA	10 mA	8 mA	10 mA
Gas Flow Rate	1.6 L/min.	2 L/min.	2.8 L/min.	1.8 L/min.	2 L/min.
Flame Height	7 mm	7 mm	9 mm	7 mm	7 mm
Flame Type	Air-Acetylene	Air-Acetylene	Air-Acetylene	Air-Acetylene	Air-Acetylene

4.8. Analytical parameters of AAS device analysis method

Table 3. Analytical parameters of the AAS analysis method for simulated body fluid mediums

Simulated Saliva Fluid Medium					
Element	Calibration Range (mg/L)	Calibration Equation	r ²	LOD (mg/L)	LOQ (mg/L)
Na	1-25	y = 0.020524x - 0.014022	0.998	0.291	0.961
K	5-20	y = 0.011929x - 0.028133	0.984	0.576	1.902
Pb	5-25	y = 0.00018800x + 0.0028400	0.991	0.930	3.071
Cr	2.5-10	y = 0.00018571x - 0.0013500	0.999	0.754	2.489
Fe	1-10	y = 0.00077692x - 0.026200	0.986	0.299	0.985
Cu	1-10	y = 0.0012497x + 0.00044146	0.995	0.274	0.903
Zn	1-10	y = 0.0046116x - 0.18944	0.992	0.265	0.875
Ni	1-10	y = 0.00044696x - 0.00072588	0.980	0.297	0.981
Mn	1-10	y = 0.0012176x - 0.046832	0.987	0.297	0.980
Cd	1-10	y = 0.0042554x - 0.15507	0.980	0.250	0.827
Simulated Gastric Fluid Medium					
Element	Calibration Range (mg/L)	Calibration Equation	r ²	LOD (mg/L)	LOQ (mg/L)
Na	1-20	y = 0.032363x + 0.0061130	0.993	0.201	0.664
K	1-15	y = 0.033361x - 0.029176	0.984	0.206	0.680
Pb	5-25	y = 0.00018835x - 0.00011785	0.997	0.929	3.065
Fe	2.5-20	y = 0.00084000x - 0.0018200	0.991	0.542	1.787
Ni	2.5-20	y = 0.00046107x + 0.0014511	0.992	0.449	1.482
Cr	2.5-20	y = 0.00030634x + 2.8495x10 ⁻⁵	0.987	0.536	1.770
Cu	1-20	y = 0.0013690x + 5.5511x10 ⁻⁵	0.997	0.542	1.787
Zn	1-20	y = 0.0048959x + 0.0025355	0.996	0.291	0.960
Mn	1-20	y = 0.0013626x - 0.0016739	0.998	0.280	0.924
Cd	1-20	y = 0.0042450x - 0.0067926	0.991	0.251	0.829
Simulated Intestinal Fluid Medium					
Element	Calibration Range (mg/L)	Calibration Equation	r ²	LOD (mg/L)	LOQ (mg/L)
Na	5-50	y = 0.0088680x + 0.0057400	0.996	1.116	3.683
K	5-100	y = 0.0052084x - 0.0072133	0.998	1.320	4.356
Fe	2.5-20	y = 0.00081907x + 0.00014860	0.998	0.711	2.346
Cr	2.5-20	y = 0.000551486x - 0.00048754	0.995	0.620	2.047
Cu	1-20	y = 0.0015262x - 0.00041378	0.999	0.281	0.929
Zn	1-20	y = 0.0053640x + 0.0029421	0.998	0.291	0.959
Ni	1-20	y = 0.00099782x - 0.0012382	0.995	0.207	0.685
Mn	0.5-20	y = 0.0019676x - 0.00062291	0.999	0.145	0.479
Cd	0.5-20	y = 0.0047856x + 0.00027872	0.998	0.124	0.410
Pb	0.5-15	y = 0.012642x + 0.0010538	0.999	0.091	0.300

4.9. Statistical method

The Minitab 17.1.0 statistics software was used to perform all statistical calculations. The PCA and HCA analysis, which are multivariate data analytic methodologies, were used in this study, to analyze *Salvia* species samples against 20 different variables.

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