







# Safety Data on *Hypericum perforatum* L. products: Phenolic profiles, antioxidant activity, and trace element assessment

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**ABSTRACT:** The use of plants for medicinal purposes, dating back to primitive healthcare practices, has evolved into traditional medicine, with significant growth in the herbal medicine market globally. Despite the widespread use of herbal products, especially in developing countries, concerns about their safety and quality persist. Issues like contamination with pathogens and heavy metals, and adulteration with cheaper substances, pose risks to human health. The quality of herbal medicines is influenced by factors like growth conditions, harvesting methods, and processing. *Hypericum perforatum* L. (St. John's wort), known for its antidepressant properties, is a popular herbal medicine. This study aims to provide safety data on *H. perforatum* products in Türkiye by analyzing the phenolic substance profiles, total phenolic content, antioxidant status, and heavy metal and mineral amounts in preparations from five different brands which were purchased randomly from pharmacies and herbalists using LC-HR/MS, DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, Folin-Ciocalteu method, Total Antioxidant Status (TAS), Total Oxidant Status (TOS), Oxidative Stress Index (OSI) methods, and ICP-OES. The analysis of *H. perforatum* samples (H5 and H2) for TAS, TOS, OSI, DPPH radical scavenging, and total phenolic content revealed significant differences in trace elements. H5 showed high antioxidant and radical scavenging activities, with notably high iron levels, while H2, with lower antioxidant but high phenolic content, had elevated zinc and copper levels. These findings suggest a link between trace elements and phytotherapeutic effects. With the increasing use of herbal products, this study underscores the growing need for stringent quality control in herbal medicine, particularly in commercially available *H. perforatum* products.

**KEYWORDS:** *Hypericum perforatum*; total antioxidant status; total oxidant status; trace elements; LC-HR/MS; ICP-OES; quality control in herbal products

## 1. INTRODUCTION

The use of plants for medicinal purposes is the oldest kind of healthcare [1]. The primitive people distinguished useful plants with beneficial effects on health by using the trial-and-error method. This gathered rich knowledge has been gradually passed on to generations; thus, the term, traditional medicine, has arisen [2].

In the 18th century, the basis of modern medicinal drugs, such as aspirin, morphine, digitoxin, and quinine, were synthesized through these plants used traditionally with the developing technology [3]. After the development of modern medicine, synthetic products gradually expanded, and they were preferred over the direct use of medicinal plants in modern

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medicine [2]. However, concerns over toxicity and iatrogenic consequences of conventional medicine in the 1960s led to a quest for safer and more affordable medications to promote health. Additionally, the World Health Organization (WHO) encouraged developing countries which does not access modern medicine, to adopt traditional medicine to address requirements. As a result, the trend worldwide has changed from synthetic to natural medications [4]. Today, approximately 80% of the world's population uses herbal medicine as their primary form of healthcare, particularly in underdeveloped countries. Additionally, the use of herbal products as prescription medications has suddenly increased in developed countries like France and Germany [3].

Moreover, the use of herbal products among people from all ages is increasing exponentially worldwide. In 2020, the herbal medicine market was expanded to US \$ 98.60 billion globally, and it has been growing steadily since then. Besides, it is projected to reach US \$ 391.22 Billion by 2028 [5].

This massive growth in the use of herbal products inevitably brings serious safety concerns, especially the quality control of herbal medicines is considered as the main obstacle [6]. Unfortunately, under-regulated quality of herbal medicines is common, and this lack of quality can be very harmful to human health because of two primary issues: Contamination and adulteration. Contamination with pathogens and heavy metals, and adulteration with cheaper substances and conventional medications are threatening both the efficacy and safety of herbal drugs. Extremely serious adverse effects including agranulocytosis, Cushing syndrome, coma, over-anticoagulation, gastro-intestinal bleeding, and arrhythmias were reported to occur because of adulterants in herbal products [7].

The quality of herbal products is influenced by various factors including the growth conditions of the plant, such as soil quality and climatic conditions, the use of herbicides and pesticides, their chemical composition, and the methods and timing of harvesting, drying, extraction, and processing [8]. The intentional addition of undeclared chemical or synthetic substances, substitution with different plant species for cost manipulation, deliberate inclusion of foreign substances [9], the presence of toxic heavy metals exceeding safe limits [10], and herbicide or pesticide residues in plant materials have raised significant concerns regarding the safety and effectiveness of plant-based products [11].

Contamination with heavy metals can occur through industrial and agricultural sources. Industrial contamination often happens during transportation and manufacturing, particularly under substandard conditions, leading to product contamination. Agricultural contamination involves the absorption of pollutants from the air and contaminated water by plant roots [12]. Heavy metals, due to their low excretion rates through the kidneys, can accumulate in the body and become harmful, even at low concentrations. While metals like zinc, copper, iron, manganese, and chromium are essential for human physiological and biological functions, excessive intake beyond permissible limits can be toxic.

The testing for safety, efficacy, and quality of herbal medicines is challenging due to the complex nature of these products, issues with authentication, and the absence of adequate standardization methods [13].

*Hypericum perforatum* L. (Hypericaceae), known as St. John's wort, is an herbaceous perennial plant native to western Asia, Europe, and northern Africa. Due to its many uses, including the treatment of skin issues like burn wound healing, stomach ulcers, biliary disorders, bronchial and genitourinary system inflammation, colds, migraines, and headaches, as well as diabetes mellitus and obesity, it is a very well-liked traditional herbal medicine. The usefulness of this plant in the treatment of mild to severe depression, however, can be credited with its popularity [14].

Being renowned for their antidepressant activity, *H. perforatum* extracts are among the most-selling herbal products globally. Preparations made with *H. perforatum* had made up as much as 25% of all prescriptions prescribed by doctors to treat depression in some countries, and *H. perforatum* containing capsules and tablets can be found on the shelves of almost every

herbal store and pharmacy) [15, 16].

To provide safety data on *H. perforatum* products in Türkiye, in this study, the phenolic substance profiles of the *H. perforatum* preparations of five different brands which were purchased randomly from pharmacies and herbalists were identified by LC-HR/MS. Total phenolic quantification of the samples were analyzed by the Folin-Ciocalteu method, and the total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress indices (OSI) of the samples were determined. Antioxidant activity of the samples was investigated by DPPH (1,1-diphenyl-2-picrylhydrazyl) method, heavy metal and mineral amounts were analyzed by ICP-OES (Inductively Coupled Plasma- Optical Emission Spectrometry).

## 2. RESULTS

### 2.1. LC-HR/MS analysis

In the LC-HR/MS method, the standard substances utilized include fumaric acid, herniarin, caffeic acid, chrysin, apigenin, emodin, naringenin, isosakuranetin, acacetin, luteolin, kaempferol, scutellarein, dihydrokaempferol, (-)-epicatechin, (+)-catechin, hispidulin, rhamnocitrin, (+)-trans taxifolin, quercetin, (-)-epigallocatechin, 3-O-methyl quercetin, nepetin, penduletin, eupatilin, rosmarinic acid, apigenin-7-glucoside, astragalin, kurentin, quercetin, (-)-epigallocatechin gallate, myricitrin, hyperoside, hederagenin, nepetin-7-glucoside, kullaic acid, naringin, luteolin-7-rutinoside, hesperidin, rutin, and verbascoside. But only hiperoside and naringenin were detected.

According to the results, the hyperoside content of H2 was the highest. H3 had the highest naringenin content but there was not much difference between the samples in terms of naringenin content (Table 1).

**Table 1.** LC-HR/MS analysis results

	Hyperoside (ppm)	Naringenin (ppm)
m/z	463.0882	271.0612
Ionisation Mode	Negative	Negative
Linear Range	0.10-7	0.10-7
H1	0.26	0.10
H2	1.25	0.10
H3	<LOD	0.14
H4	0.29	0.11
H5	0.18	0.13
LOD/LOQ	0.33/1.09	0.20/0.67
U % (k=2)	3.01	4.15

### 2.2. Determination of DPPH free radical scavenging activity, total phenolic content, total antioxidant status, total oxidant status, and oxidative stress index

The antioxidant activities of the samples and standard antioxidant substances (BHT and Vit. C) were assessed using DPPH radical scavenging assay. The antioxidant activities of the samples were compared with BHT and Vit. C. The findings indicated that, Vit. C exhibited the most potent radical scavenging effect. Following Vit C, the order of effectiveness in terms of radical scavenging was determined to be BHT, H5, H1, H2, H4, and H3 (Table 2).

Upon examining the total phenolic content determined as Gallic Acid Equivalents (GAE), it was observed that the phenolic content in samples H1, H4, and H2 was higher compared to the other samples. It was also noted that the phenolic content in the H3 sample was significantly lower than that in the others.

The findings predominantly indicate significant variations in the Oxidative OSI levels among the samples, primarily due to substantial differences in their TOS levels rather than TAS levels. It was determined that the TAS levels in samples from H5 and H1 were statistically higher ( $p < 0.05$ ) compared to the other samples.

### 2.3 Mineral matter levels

In the conducted analyses of mineral content, it was observed that the levels of elements such as Sb, As, Be, Cd, Cr, Co, Pb, Mo, Se, Ti, Ag, Al, B, Ba, Bi, Ga, Ca, and K were out of the working range of the study. The elements detectable within the working range are presented in Table 3.

**Table 2.** DPPH free radical scavenging activity, total phenolic content, total antioxidant status, total oxidant status, and oxidative stress index results

Samples	DPPH free radical scavenging activity (Inhibition rate %)	Total phenolic content (mg GAE/g extract)	Total antioxidant status (TAS) (mmol Trolox Equiv./L)	Total oxidant status (TOS) ( $\mu\text{mol H}_2\text{O}_2$ Equiv./L)	Oxidative stress index (OSI) (Arbitrary units)
H1	88.09 $\pm$ 0.20 <sup>c</sup>	6.28 $\pm$ 0.33 <sup>c,d</sup>	2.36 $\pm$ 0.05 <sup>c</sup>	105.26 $\pm$ 5.72 <sup>b</sup>	44.56 $\pm$ 3.02 <sup>b</sup>
H2	84.03 $\pm$ 0.17 <sup>b</sup>	5.41 $\pm$ 1.07 <sup>c</sup>	2.05 $\pm$ 0.02 <sup>a,b</sup>	95.01 $\pm$ 15.80 <sup>b</sup>	46.30 $\pm$ 8.20 <sup>b</sup>
H3	59.76 $\pm$ 1.33 <sup>a</sup>	0.96 $\pm$ 0.07 <sup>a</sup>	2.16 $\pm$ 0.04 <sup>b</sup>	16.59 $\pm$ 3.21 <sup>a</sup>	7.71 $\pm$ 1.64 <sup>a</sup>
H4	84.72 $\pm$ 0.62 <sup>b</sup>	6.51 $\pm$ 0.41 <sup>d</sup>	1.94 $\pm$ 0.12 <sup>a</sup>	97.16 $\pm$ 14.16 <sup>b</sup>	50.41 $\pm$ 10.05 <sup>b</sup>
H5	88.95 $\pm$ 1.33 <sup>c</sup>	4.04 $\pm$ 0.45 <sup>b</sup>	2.41 $\pm$ 0.08 <sup>c</sup>	26.35 $\pm$ 2.00 <sup>a</sup>	10.93 $\pm$ 0.87 <sup>a</sup>
BHT	88.45 $\pm$ 0.29 <sup>c</sup>	-	-	-	-
Vit. C	92.01 $\pm$ 1.78 <sup>d</sup>	-	-	-	-
P	0.000	0.000	0.000	0.000	0.000

The data are presented as mean  $\pm$  standard deviation (n=3).

<sup>a, b, c, d</sup>: In the same column, means carrying different superscript letters are statistically significantly different (p < 0.05).

**Table 3.** Mineral matter levels

Samples	Cu (ppm)	Fe (ppm)	Li (ppm)	Mg (ppm)	Sr (ppm)	V (ppm)	Zn (ppm)	Ni (ppm)	Mn (ppm)
H1	5.97 $\pm$ 0.53 <sup>a</sup>	5.13 $\pm$ 0.90 <sup>a</sup>	0.59 $\pm$ 0.02 <sup>a</sup>	164.25 $\pm$ 8.46 <sup>a</sup>	2.73 $\pm$ 0.098 <sup>a</sup>	-	17.63 $\pm$ 1.99 <sup>a</sup>	1.02 $\pm$ 0.27 <sup>a</sup>	40.98 $\pm$ 3.26 <sup>a</sup>
H2	21.69 $\pm$ 0.66 <sup>b</sup>	50.25 $\pm$ 14.07 <sup>a</sup>	7.42 $\pm$ 1.47 <sup>c</sup>	723.05 $\pm$ 63.91 <sup>d</sup>	37.72 $\pm$ 5.27 <sup>d</sup>	4.42 $\pm$ 0.51 <sup>b</sup>	44.89 $\pm$ 9.46 <sup>b</sup>	-	-
H3	-	138.48 $\pm$ 12.34 <sup>a,b</sup>	5.55 $\pm$ 0.55 <sup>b</sup>	404.12 $\pm$ 7.78 <sup>b</sup>	27.72 $\pm$ 1.46 <sup>c</sup>	3.46 $\pm$ 0.19 <sup>a</sup>	31.81 $\pm$ 25.58 <sup>a,b</sup>	-	-
H4	19.98 $\pm$ 1.46 <sup>b</sup>	19.24 $\pm$ 3.66 <sup>a</sup>	5.38 $\pm$ 0.26 <sup>b</sup>	628.17 $\pm$ 29.24 <sup>c</sup>	20.84 $\pm$ 0.47 <sup>b</sup>	4.42 $\pm$ 0.39 <sup>a,b</sup>	46.44 $\pm$ 6.11 <sup>b</sup>	-	-
H5	-	266.25 $\pm$ 162.49 <sup>b</sup>	5.81 $\pm$ 0.98 <sup>b</sup>	713.38 $\pm$ 30.47 <sup>d</sup>	33.06 $\pm$ 2.73 <sup>d</sup>	4.85 $\pm$ 0.59 <sup>b</sup>	27.40 $\pm$ 1.91 <sup>a,b</sup>	-	16.04 $\pm$ 1.45 <sup>b</sup>

The data are presented as mean  $\pm$  standard deviation (n=3).

<sup>a, b, c, d</sup>: In the same column, means carrying different superscript letters are statistically significantly different (p < 0.05).

### 3. DISCUSSION

LC-HR/MS analysis showed that the samples were containing only hyperoside and naringenin among the 40 standards. On the other hands, Ersoy and colleagues examined three different *Hypericum* species using LC-MS/MS, it was found that the methanol extract of *H. perforatum* contained naringenin at a rate of 4.64  $\pm$  0.002  $\mu\text{g/g}$  [16]. In the other research conducted by Kelebek and colleagues, which illuminated the phenolic profiles of *H. perforatum* teas prepared using three different methods through LC-DAD-ESI-MS/MS, the hyperoside content in three different teas was determined to be 5.15 $\pm$ 0.19 mg/L, 5.73 $\pm$ 0.15 mg/L, and 6.16 $\pm$ 0.208 mg/L, respectively [17]. These values were observed to be higher than those in all the samples.

Oxidative damage is implicated in a wide range of diseases and physiological disorders, including allergies, cancers, cardiac and vascular disorders, genetic and metabolic disorders, infectious diseases, neurodegenerative diseases, and ophthalmological problems [18, 19]. *H. perforatum*, with its high antioxidant

activity, may play a significant role in the treatment of neurodegenerative diseases such as Alzheimer's, Parkinson's, and stroke [19]. In a study by Zou et al., *H. perforatum* was identified as a potent antioxidant due to its rich flavonoid content [20]. Additionally, the quantity of phenolic compounds present in the composition of herbal extracts is indicative of the extract's antioxidant potential [21]. The results showed that five different samples exhibited antioxidant effects nearly equivalent to BHT and comparable to Vit. C.

These results are consistent with the comparisons made based on TAS, TOS, and OSI levels. The sample with the lowest radical scavenging effect, H3 (59.76±1.33%), could be attributed to its very low total phenolic content, aligning with the findings from the phenolic content determination section.

Although medicinal plants contain antioxidant compounds, they also possess oxidants in certain amounts. Lower levels of oxidants in a plant intended for phytotherapeutic use facilitate easier tolerance by the organism utilizing it for therapeutic purposes. This aspect is crucial for the more pronounced manifestation of the antioxidant effects of the plant's active components. Upon examining the TOS levels of the analyzed samples, it was noted that samples H2, H4, and H1 exhibited statistically higher TOS levels compared to others, suggesting that the oxidants present might adversely affect antioxidant activity.

Indeed, OSI values, derived from dividing TOS levels by TAS levels and considered an indicator of oxidative stress, were high for these three samples. This implies that their antioxidant activities might be lower compared to others. Conversely, samples H3 and H5, with lower OSI values, are likely to exhibit higher antioxidant activities.

Cu, Fe, Mn, and Zn are essential trace elements performing numerous vital biological functions. Cu and Zn serve as components of cytosolic superoxide dismutase (SOD1) [22, 23], while Mn is integral to mitochondrial superoxide dismutase (SOD2) [24]. Both SOD1 and SOD2 play a critical role in maintaining the oxidant-antioxidant balance and averting oxidative stress. Additionally, Cu, transported by the endogenous antioxidant ceruloplasmin, contributes significantly to bone and tooth formation and is involved in iron metabolism [22]. Zn, apart from its antioxidative functions, influences endocrine system health, particularly impacting thyroid and pancreatic hormone metabolism [25]. Fe is known for its role in metabolism, oxidative stress, and antioxidants, being a key player in various pathophysiological processes. Catalase, an enzymatic antioxidant comprising four polypeptide tetramers with 500 amino acid residues each, contains four porphyrin heme (Fe) groups, enabling the enzyme to convert hydrogen peroxide into water [26, 27].

In this context, the five samples rich in Cu, Fe, Mn, and Zn could be significant nutritional sources. The WHO report on heavy metals in herbal medicines identifies As, Pb, Cd, Cr, Hg, Cu, and Li as toxic metals, setting maximum permissible limits. In the five samples tested, no toxic elements other than Cu and Li were detected, either due to their absence or levels being outside the operational range. The detected Cu content in the samples exceeded the WHO limit of 150 ppm, while Li was below 10 ppm [28-30], aligning with the established standards for toxic element content.

#### 4. CONCLUSION

In examining the outcomes of the analysis (TAS, TOS, OSI, DPPH radical scavenging, and total phenolic acid content) concerning trace elements, it is evident that samples H5 and H2 stand out compared to other samples. The H5 sample, which exhibits superior antioxidant activity, an effective radical scavenging capacity, and a high total phenolic content, is notable for its significantly higher Fe level. Although its Zn level is not statistically different and is lower than in groups with the highest zinc levels, its Mn level is considerable, despite Cu being below the detection limit. This suggests that the observed phytotherapeutic effects might be attributed to the presence of trace elements, aligning with the biochemical findings. In the case of the H2 sample, which shows lower antioxidant activity but a high content of phenolic substances and radical scavenging effect, the elevated levels of Zn and Cu are believed to enhance its radical scavenging properties compared to other samples. Furthermore, the concentrations of heavy metals in these samples fall outside their operational range, which could imply a reduced risk of heavy metal toxicity.

As the consumption of herbal products continues to grow each day, the importance of maintaining their quality and implementing stringent quality control protocols is becoming more vital. While numerous studies highlighting the necessity of these quality measures exist in the literature, this research focuses on comparing commercially available *H. perforatum* preparations. In line with the escalating use of herbal products, there is a corresponding need to enhance the quality control procedures for these products.



## 5. MATERIALS AND METHODS

### 5.1. Plant Material

Five different products (H1, H2, H3, H4, H5) containing *H. perforatum* extract were purchased from different randomized herbalists and pharmacies in Istanbul.

### 5.2. Chemicals and Kits

Kits for TAS and TOS levels were purchased from Rel Assay Diagnostics, Türkiye. All other chemicals were purchased from Sigma-Aldrich (Sigma-Aldrich Chemical Co. St. Louis, MO, USA).

### 5.3. Extraction

Samples were crushed in a porcelain mortar. From these homogenized samples, 100 mg was weighed and placed into a tube. Methanol (Sigma) was added in a 1:100 (w/v) ratio (10 mL). It was mixed with a vortex. Subsequently, the samples were incubated in a dark environment at +4 °C for 24 hours. After the incubation, the sample-methanol mixtures were subjected to sonication 10 times at an ultrasonic frequency of 20 kHz in ice. Each sonication was applied for 20 seconds followed by a 1-minute rest for the sonicator (Bandelin, UW 2070). This aimed to further break down the plant at the cellular level, making the samples even more homogeneous. The methanol-sample suspension formed by sonication was centrifuged at +4 °C and 8500 rpm to obtain the supernatants of the samples.

### 5.4. LC-HR/MS Analysis

A previously validated method [31] was carried out to identify the phenolic contents of samples. The extracts were diluted to 10 ppm with methanol, then, an internal standard solution of 100 ppm was added to achieve a final concentration of 3 ppm, and the samples were filtered through a 0.45 µg filter. LC-HR/MS analysis was performed by using 40 standard phenolic compound and Thermo ORBITRAP Q-EXACTIVE system. The separation was achieved using a Fortis UniverSil C18 (150 mm × 3 mm, 3 µm) analytical column. During the column oven temperature was set at 40 °C. The elution gradient contains %1 formic acid-ultrapure water (mobile phase A), and %1 formic acid-methanol (mobile phase B). The following LC gradient elution program was applied for optimum separation: 50% B (0-1 min), 50-100% B (1-3 min), 100% B (3-6 min), 100-50% B (6-7 min), 50% B (7-10 min). The system delivered a constant solvent flow of 0.35 mL/min and the volume of injection was 2 µL.

### 5.5. Determination of DPPH free radical scavenging activity, total phenolic content, total antioxidant status, total oxidant status and oxidative stress index

DPPH radical scavenging activities of samples were determined in accordance with the Brand-Williams method [32], which is based on the principle of scavenging the DPPH radical. First, the absorbance values at 517 nm of DPPH radical solutions prepared at five different concentrations of 7.5, 15, 30, 60, 120 µM were described, and a calibration curve was drawn. Then, 3.9 mL of DPPH solution was added to 0.1 mL of the solutions prepared with extracts and standard antioxidant substances (BHT and Vitamin C) and stirred. Each mixture was kept in the dark for 30 min and the absorbance of the color formed as a result of the reaction was measured at 517 nm in a spectrometer. The results were calculated using the calibration curve of DPPH ( $R^2=0.9998$ ), and the inhibition rates of the samples were determined using this formula: DPPH Inhibition rate (%) =  $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$ . Total phenolic contents (TPC) of the samples were determined using Folin-Ciocalteu method described by Ainsworth and Gillespie [33]. The absorbance values of the samples were determined using this formula: Absorbance =  $0.0029 \text{ gallic acid } (\mu\text{g}) + 0.3113$ ,  $R^2=0.9817$ . The results were expressed in terms of gallic acid equivalents (GAEs), measured in micrograms. Total antioxidant status (TAS) levels were measured using commercially available kits from Rel Assay Diagnostics in Türkiye according to a method. The results were expressed as millimol Trolox equivalents per liter (mmol Trolox equiv./L). Also, the total oxidant status (TOS) levels were determined using a novel automated measurement method. The results were calibrated with hydrogen peroxide and expressed in terms of micromolar hydrogen peroxide equivalent per liter ( $\mu\text{mol H}_2\text{O}_2 \text{ equiv./L}$ ). Oxidative Stress Index (OSI) was computed using the Total Antioxidant Status (TAS) and Total Oxidant Status (TOS) data obtained from the analyses. As described in the kit protocols (Rel Assay), the OSI was calculated using the formula  $\text{OSI} = (\text{TOS} / \text{TAS})$  [34].

## 5.6. Mineral Matter Levels

In this study, a microwave oven combustion method to decompose organic components and solubilize inorganic components in the samples. Initially, 0.5 g of each raw sample was placed into Teflon vessels. To these samples, a mixture of acids was added, consisting of 3 mL nitric acid, 1 mL hydrogen peroxide, and 0.5 mL perchloric acid. The vessels were then sealed and placed in a microwave oven (Berghof Speedwave two) for a specific heating regimen. The heating protocol involved maintaining the samples at 75 °C /5 mins, 160 °C /15 mins, and 75 °C /5 mins. Upon cooling to room temperature, the contents of the vessels were transferred to 10 mL volumetric flasks and diluted to the mark with 18.2 MΩcm ultrapure water. The concentrations of minor and major elements in these samples were subsequently measured using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES; Spectro Genesis, Germany). For the calibration of the ICP-OES, multi-element standards from Merck (ICP multi-element standard solution XVI and IV) were utilized. Following the microwave oven wet combustion process, the elemental levels in the samples were determined and expressed in ppm, in accordance with the methodology outlined by [35].

## 5.7. Statistical Analysis

The obtained data were presented as mean ± standard deviation. The SPSS 18 software package was used for data analysis. The statistical differences ( $p < 0.05$ ) between the data obtained from the analysis of *H. perforatum* sourced from different herbalists and the capsules claimed to be derived from it were determined using one-way analysis of variance (ANOVA). Where statistical differences were identified, the Duncan post hoc test was used to determine which species of plant showed statistically significant differences ( $p < 0.05$ ). In a parameter analyzed, the statistical differences resulting from comparisons between plant samples were indicated in the upper right corner of the data with superscript symbols ("a, b, c, etc.").

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