Research Article

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Studies on the chemical composition of plants used in traditional medicine in Congo

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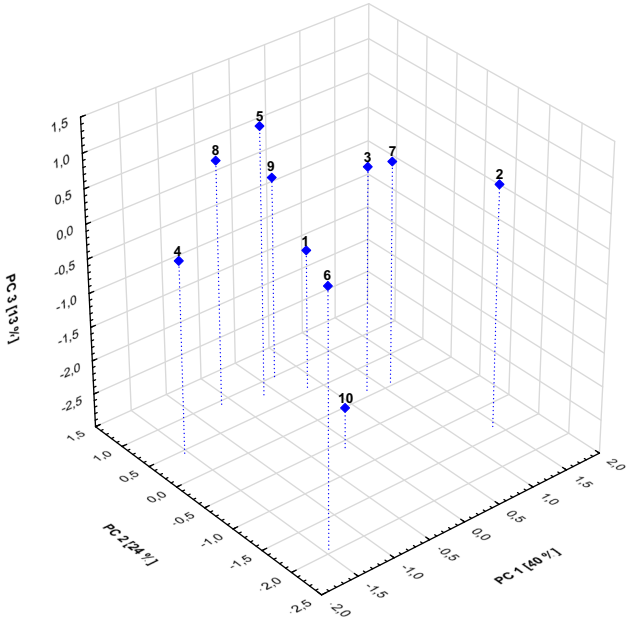
Abstract: The knowledge of the chemical composition of herbs used medicinally in Africa is relatively low, and at the same time, the growing interest in alternative medicine prompts scientists to search for justification for the use of various plants. Due to these, the aim of the study was to analyze ten botanical species of medicinal plants originating from Congo to determine the contents of nonmetals (P, Se), metallic elements (Cu, Zn, Fe, Mn, Ca, Mg, Na, K, Cd, Pb, Cr, Co, Al), phenolic compounds, and L(+) ascorbic acid and antioxidant activities. To prepare plant samples for quantitative analysis by flame atomic absorption spectrometry (FAAS) and inductively coupled plasma-optical emission spectroscopy, the microwave digestion was applied. The contents of phenolic acids, flavonoids, polyphenolic compounds, and vitamin C were assayed in extracts of medicinal plants spectrophotometrically, while the antioxidant activity was determined by ferric reducing antioxidant power (FRAP), 2,2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH), and 2,2'-Azino-bis-3-ethylbanzothiazoline-6-sulfonic acid (ABTS) techniques. The studies showed that the medicinal plants from Congo differed to a high degree. Principal component analysis demonstrated that the concentrations of Cu, Fe, Mn, and also phenolic compounds and antioxidant activity had the highest impact on sample's differentiation. The relationship between the type of plant material (bark, root, or leaf) and its chemical composition was noticed, too.

Keywords: folk medicine, Congo, metallic elements, phenolics, statistical methods



Graphical abstract

1 Introduction

The use of plants for medicinal purposes in Africa is very popular [1,2]. Among them, there are herbal remedies against diseases such as anemia, fever, infections, pains of various origins, sexual malfunctions, etc. It was recently reported that in spite of extensive research on African plants used in medicine, their therapeutic properties were not completely evaluated [3]. For example, medicinal plants from Africa such as *Eriocephalus punctulatus, Hypoxis hemerocallidea, Dicoma anomala, Xysmalobium undulatum, Morella serrata*, *Gazania krebsiana*, and many others can be used against diabetes, as demonstrated in recent studies [4]. Other applications of herbal drugs originating from the African continent include their anti-fungal activity [5], antimicrobial actions used for the treatment of respiratory infections [6], and their potential use for the prevention of rheumatoid arthritis [7]. The medical activity of natural drugs can also be related to the content of metallic and nonmetallic elements

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in the plants. There are several studies on elements in African medicinal plants available in the literature. For example, Steenkamp et al. [8] analyzed 82 plant remedies from South Africa for their metallic element contents. The researchers have found that some of the bark under analysis had arsenic concentrations higher than 0.3 mg/kg, which could pose a health risk for people using them. As for the other toxic metals determined in the studied material, such as Pb, Bi, Ba, and Ni, they were below the recommended daily dose limits [8]. Thus, the authors suggested that their study has indicated that metal toxicity from plant-based traditional remedies of South Africa appeared not to be a risk factor for local people who use them in the treatment.

On the other hand, Okem et al. [9] in their investigations on South African medicinal plants found that the levels of As and Hg were above the limits set by WHO in most of the investigated samples. High concentrations of the contaminants were probably caused by anthropogenic activity, especially because some medicinal plants have the ability to accumulate heavy metals from the polluted soils where the plants had grown. Another hazardous metallic element that is often analyzed in African plants is lead. Its elevated level was determined in plants growing in Nigeria and Senegal, and the reason for Pb poisoning in children was artisanal gold mining and from battery recycling [10].

Most of the studies on the chemical composition of medicinal plants was performed in South Africa but also in Equatorial African countries, such as the Democratic Republic of Congo (DR Congo). However, there is scarce knowledge of plants used in traditional medicine in the neighboring country – Congo. Taking all the above into consideration, the aim of the study was to analyze ten botanicals species of medicinal plants originating from Congo to determine the concentrations of non-metals (P, Se), metallic elements (Cu, Zn, Fe, Mn, Ca, Mg, Na, K, Cd, Pb, Cr, Co, Al), phenolic compounds, and L(+) ascorbic acid in them and antioxidant activity in order to classify medicinal plants as rich or poor in the studied analytes.

2 Materials and methods

2.1 Preparation of samples prior to analysis

Medicinal plants compiled in Table 1 were collected and identified by Dr Edmond Sylvestre Miabiangana in the area of Brazzaville, Congo. After drying the medicinal

Table 1: The list of folk medicines originating from Congo

Sample No.	Latin name	Botanical family	Origin, place of harvest	Plant part	Medicinal use
1	*Pausinystalia johimbe* (K. Schum.) Pierre ex Beille	Rubiaceae	Forest near Djoumouna river	Trunk bark	Aphrodisiac, vasodilator of the genitals and anti-fatigue
2	*Alchornea cordifolia* (Schumach. and Thonn.) Müll. Arg.	Euphorbiaceae	Garden in Mbouono district of Brazzaville, near Congo river	Leaf	Used against bronchitis in children, against coughs
3	*Syzygium brazzavillense* Aubrèv. and Pellegr	Myrtaceae	Young forest near Djoumouna river	Trunk bark	Recommended against anemia
4	*Quassia africana* (Baill.) Baill.	Simaroubaceae	Thick forest near Djoumouna river	Root	Against malarial fevers and dysentery
5	*Zanthoxylum gilletii* (De Wild.) P. G. Waterman	Rutaceae	Thick forest near Djoumouna river	Trunk bark	Advised in severe anemia crisis
6	*Mitragyna stipulosa* (DC.) Kuntze	Rubiaceae	Former swamp forest near Congo river in Mbouono district of Brazzaville	Trunk bark	Advised against febrile states
7	*Pentaclethra macrophylla* Benth.	Fabaceae	Forest near Brazzaville	Trunk bark	Indicated for lower back and intercostal pain (low back pain)
8	*Sarcocephalus latifolius* (Sm.) E.A. Bruce	Rubiaceae	Savanna near Djoumouna river	Trunk bark	Advised in the treatment of urogenital infections and against fever
9	*Ochna afzelii* R. Br. ex Oliv.	Ochnaceae	Shrub savanna in Mayala – Kombe, near Djoumouna river	Trunk bark	Recommended against anemia
10	*Garcinia huillensis* Welw. Ex Oliv.	Clusiaceae	Savanna near Touo forest, Kibosi	Root	Used for pelvic pain and as aphrodisiac for men

plant samples were transported to the Department of Analytical Chemistry at the Medical University of Gdansk, Poland, then ground using a sample preparation mill (Knifetec 1095, Foss Tecator, Sweden), and stored in plastic containers in a dry and dark place prior to analysis.

About 0.5 g of samples was then digested using a microwave unit (Jupiter, Sineo, China) applying a four-stage digestion program: step I – 150°C (10 min), step II – 160°C (5 min), step III – 180°C (5 min), and step IV – 190°C (15 min). The obtained digests were transferred into 50 mL volumetric flasks and the volume was done with distilled water.

To measure the contents of phenolic acids, flavonoids, polyphenolic compounds, and ascorbic acid, as well as determine the antioxidant activity, aqueous extracts were prepared by pouring 0.5 g of plant material in 50 mL of boiling redistilled water. After filtration, the extracts were transferred to 50 mL volumetric flasks, and the volume was made with redistilled water. Redistilled water was obtained using a distillation apparatus (Heraeus Quarzglas, Germany).

2.2 Determination of metallic elements

The contents of essential elements, Fe, Mn, Zn, Cu, Cd, and Pb, were determined by the flame atomic absorption technique (SpectrAA 250 Plus, Varian, Australia) using standard analytical procedures and an external calibration method. An air–acetylene mixture was used during the measurements along with the following analytical wavelengths (nm) for the particular metallic elements: 248.3 (Fe), 279.5 (Mn), 213.9 (Zn), 324.8 (Cu), 228.8 (Cd), and 217.0 (Pb). Chromium, Co, Al, and Se were assayed by the inductively coupled plasma-optical emission spectroscopy (ICP-OES) technique using a PerkinElmer Optima 7000DV spectrometer (Perkin-Elmer, Waltham, MA, USA).

Phosphorus was determined in the digests of medicinal plants by a spectrophotometric technique using a molybdenum blue reaction [11] at 650 nm as the analytical wavelength and an external calibration curve.

2.3 Total phenolic content (TPC)

The TPC of the Congo plant extracts was determined using the Folin–Ciocalteu method, as previously described by Singleton [12]. The absorbances of the extracts from Congo folk medicines were measured at 760 nm using an SP-870 Metertech UV/Vis spectrophotometer (South Korea). The gallic acid calibration curve (0.1–0.154 mg/mL) was used to express the results as milligrams of gallic acid equivalents per gram of dry mass (mg GAE/g dm).

2.4 Total flavonoid content (TFC)

The TFC of the Congo plant extracts was determined based on the method of the European Pharmacopeia [13] with some modifications. An appropriate amount of the extract was mixed with 0.1 mL of 5% (w/v) $AlCl_3$ solution. The mixture was incubated for 30 min in the dark at room temperature and the absorbance was then measured at 430 nm using an SP-870 Metertech UV/Vis spectrophotometer (South Korea). The TFC was expressed in milligrams of quercetin equivalents (QE) per gram of dry mass (mg QE/g dm) using a calibration curve obtained from quercetin standard solutions (5–50 μg/mL).

2.5 Total phenolic acid content (TPAC)

The procedure described in the Polish Pharmacopeia VI [14] was applied for TPAC determination using Arnov's reagent. An appropriate amount of the extract was mixed with 0.2 mL of 0.5 M HCl, 0.2 mL of Arnov's reagent and 0.2 mL of 1 M NaOH. The absorbance was measured at 490 nm. The results are expressed in milligrams of caffeic acid equivalents per gram of dry weight (mg CAE/g DW) based on a calibration curve registered for caffeic acid (5–40 μg/mL).

2.6 Ascorbic acid determination and antioxidant activity

The ascorbic acid content in extracts of medicinal plants from Congo was assayed based on the analytical procedure described elsewhere [15]. Antioxidant activity was determined in the extracts of plants using FRAP, DPPH, and ABTS methods, also described previously [15].

2.7 Statistical evaluation of the results

All measurements were carried out in triplicate, and the results were presented as the arithmetic mean ± standard

Table 2: Statistical evaluation of results obtained for metallic elements in CRMs by AAS

Element	Determined concentration in CRM	Precision as RSD (%)	Declared concentration in CRM	Recovery (%)	CRM
Cu	9.98 mg/kg	1.55	7.77 ± 0.53 mg/kg	128.4	MPH
Zn	29.39 mg/kg	0.68	33.5 ± 2.1 mg/kg	87.7	MPH
Fe	413.16 mg/kg	1.16	460 mg/kg	89.8	MPH
Mn	152.45 mg/kg	2.49	191 ± 12 mg/kg	79.8	MPH
P	2877.05 mg/kg	1.77	2500 mg/kg	115.1	MPH
Cd	246.80 mg/kg	3.35	199 ± 15 mg/kg	124.0	MPH
Pb	2.31 mg/kg	1.99	2.16 ± 0.23 mg/kg	107.1	MPH
Ca	2.85%	1.45	2.297%	124.1	PVTL
Mg	0.29%	3.13	0.292 ± 0.018%	101.2	MPH
Na	390.59 mg/kg	2.70	350 mg/kg	111.6	MPH
K	1.48%	0.75	1.03%	144.3	VTL

MPH = mixed polish herbs (INCT–MPH–2); VTL = virginia tobacco leaves (IC–CTA–VTL2); PVTL = polish virginia tobacco leaves (INCT–PVTL–6).

deviation (SD). Table 2 presents the statistical evaluation of the results of element's determination in terms of their precision and recovery obtained for selected certified reference materials (CRMs) using FAAS. Validation parameters for ICP-OES were published in our previous article [23]. For statistical evaluation of experimental data using principal component analysis (PCA), the Statistica 7.1 program (Tulsa, USA) was used.

Validation of methods applied for TPAC, TPC, and TFC was performed by evaluating the following parameters: linearity, limit of detection (LOD), and limit of quantification (LOQ), intra- and inter-day precision, recovery, and stability. Good linearity was found over the determined ranges for all analytes, with correlation coefficient values significantly higher than 0.980. The LOD and LOQ were calculated in accordance with the following equations: LOD = $3.3Sxy/b$ and LOQ = $10Sxy/b$, where Sxy is the SD of the response and b is the slope of the calibration curve. The values of LODs and LOQs were less than 3.5 and 13.2 μg/mL, respectively. These results show that the analytical method had excellent resolution and sensitivity. Intra-day precision was validated with a standard solution of assayed phenolic compounds three times within 1 day, while inter-day precision was validated with the same standard solution over three consecutive days. Consequently, the precision was acceptable, and the coefficient of variation values ranged from 0.6 to 1.4% and 1.2 to 2.5% for intra- and inter-day variations, respectively.

The mean recovery was found to be in the satisfactory range, 92.6–97.4%, with a relative SD of less than 4.5%. The peak areas and retention times of the determined phenolic compounds were analyzed every 8 h within 48 h for the stability test, and they were found to be quite stable, while retention CV was lower than 1.6% for peak area and 0.5% for retention time. All validation values described above were obtained for phenolic compound analysis.

3 Results and discussion

3.1 Contents of metallic elements, phosphorus, and selenium

Results of element determination in ten analyzed medicinal plants from Congo are presented in Table 3. The characteristic feature of the presented data is the fact that the arithmetic mean and the SD represent a similar range of concentrations. This is the effect that these values were obtained after measuring the elements in all analyzed samples of folk medicines from Congo, and their level was largely different. As for results obtained for P, the only non-metal element analyzed, it was found in the wide range from about 0.14 to 2.96 mg/g dm. An especially high level of P was determined in the leaf of *Alchornea cordifolia*, almost 3 mg/g dm. On the other hand, the lowest P concentrations were noticed in two plant samples – *Ochna afzelii* and *Syzygium brazzavillense*, at about 135.00 and 151.00 mg/kg dm, respectively. Other research on the phosphorus level has shown that in plants originating from the DR Congo, it was found in the range from 1258.00 to 1567.00 mg/kg dm [16], which is rather close to the highest results obtained in our study. The P level in the plant material from Congo is lower than those reported in European plants used in medicine [17,18]. The reason for this can be the different P levels

Table 3: Results of element determination in ten medicinal plants from Congo

Element	Arithmetic mean ± SD	Median	Range
Cu	21.97 ± 22.81	10.11	3.10–60.06
Zn	33.09 ± 30.41	19.51	3.00–90.13
Fe	185.15 ± 12.73	137.01	65.60–437.37
Mn	68.39 ± 6.85	32.616	14.31–211.29
P	613.36 ± 85.53	250.38	138.81-2962.18
Cd	3.87 ± 1.08	4.30	<LOD–5.55
Pb	5.32 ± 3.55	4.30	<LOD–14.40
Ca*	24.80 ± 1.74	24.66	8.10–40.87
Mg*	7.30 ± 2.50	6.61	3.95–12.24
Na*	24.15 ± 4.95	25.26	15.90–32.08
K*	14.37 ± 6.17	14.20	4.57–27.24
Cr**	5.92 ± 8.88	1.33	0.41–27.40
Co**	0.093 ± 0.079	0.073	0.025–0.294
Al**	235.09 ± 251.60	137.00	30.40–715.00
Se**	10.59 ± 12.23	6.05	1.98–42.30

The arithmetic mean (mg/kg dm) ± SD is given (n = 3).
*mg/g dm.; **determined by ICP-OES.

in soils of Africa and Europe but also anthropogenic factor cannot be neglected. The latter effect is mainly due to the use of fertilizers containing phosphorus, which can elevate the concentration of this element in plants [19].

The concentrations of Ca, Mg, Na, and K, also shown in Table 3, indicated several plants with characteristic levels of them. Calcium concentration was found to be the highest in the bark of *Zanthoxylum gilletii*, 40.49 mg/g dm, and in the leaf of *A. cordifolia*, 39.74 mg/g dm. The lowest Ca level was determined in *Q. africana*, 8.55 mg/g dm. In general, the Ca level in our study was higher than those determined by other researchers, both in plants from Africa [16] and Iran [20]. As for Mg, the richest plant material from Congo with these elements was the bark of *S. brazzavillense*, about 12 mg/g dm, as well as the bark of *Mitragyna stipulosa*, which contained almost 11 mg/g dm. Lower than the values given above, the Mg concentration was determined in plants from DR Congo, about 1.70–1.90 mg/g dm [16], but a similar Mg level was found in South African medicinal plants [8]. Sodium content was the highest in the sample of *A. cordifolia*, about 32.00 mg/g dm, and in the bark of *P. macrophylla*, 31.00 mg/g dm. Relating these values to Na levels determined by other researchers [16,20], they are higher. The contents of K varied in different samples depending on plant species. The highest K level was found in the bark of *M. stipulosa*, about 27.00 mg/g dm, and the lowest in the bark of *S. brazzavillense*, about 4.80 mg/g dm. The former value is quite similar to the concentration of K found in plants from South Africa [8] and DR Congo [16].

The contents of microelements in medicinal plants from Congo are presented in Table 3, too. Among microelements essential for human life, the order in which their concentrations (in mg/kg dm) were obtained is as follows: Fe > Mn > Zn > Cu. The plant materials with relatively high levels of these metals, such as samples *Q. africana, Garcinia huillensis, M. stipulosa,* and *A. cordifolia*, can be listed. When comparing the obtained levels of essential elements with those in the literature, it can be stated that these levels strongly depend on plant species and analyzed plant organs. For example, the level of Cu in medicinal plants in DR Congo [16] was determined in a similar range of concentrations, about 10.00–11.00 mg/kg dm. The same can be noticed for Zn, Fe, and Mn [8,16,21]. The results of essential element assays determined for plants from Congo in this study also remain in agreement with their levels in medicinal plants originating from Europe [17,18].

Table 3 also presents the results of the determination of harmful elements for humans, such as Cd, Pb, Cr, Co, and Al. Cadmium and lead were assayed by the FAAS technique, and the other elements by ICP-OES. The highest level of Cd was found in the sample of *Zanthoxylum giletii*, 4.5 mg/kg dm, and in *Q. afzelii*, 5.0 mg/kg dm. On the other hand, for plants such as *Q. africana, M. stipulosa* and *G. huillensis*, Cd was below the LOD for the FAAS technique. The level of Cd detected in the plants from Congo was however higher than that found in other African plants, for example, in Nigeria [22]. Perhaps the reason for this can be higher Cd contamination of soils near Brazzaville in Congo. Lead was determined in the highest concentration in the sample of bark from *M. stipulosa*, about 14.20 mg/kg dm. In other investigated plants, the Pb level was much lower or below the LOD. This is typical for African plants since the Pb level can vary depending on local contamination and/or anthropogenic factor. In South African plants [8], the level of Pb was determined in a lower amount in comparison with the Pb concentration in the bark of *M. stipulosa*, and in other research studies performed on plants from DR Congo [16] its level was about 1.00 mg/kg dm or lower.

Elements determined by ICP-OES represented a low range of concentrations in comparison with those assayed by FAAS. Particularly rich in Se were plants such as *M. stipulosa* (42.30 mg/kg dm), *Pausinystalia johimbe* (15.90 mg/kg dm), and *Q. africana* (15.80 mg/kg dm). The aluminum level was the highest in the sample of *Q. africana* – 678.00 mg/kg dm, about ten times higher than that detected in other medicinal plants. Chromium and Co levels were determined as the highest in the sample of *M. stipulosa*, 15.00 and 0.29 mg/kg dm, respectively.

3.2 Phenolic compound analysis

3.2.1 TPACs

The mean concentration of TPAC in all studied folk medicines from Congo is 29.87 mg/g dm (Table 4). The highest contents of phenolic acids were detected in the bark of *P. johimbe*, from 75.93 to 83.65 mg/g dm, and in the root of *G. huillensis*, from 68.43 to 72.35 mg/g dm. The lowest TPAC was found in the root of *Q. africana*, 1.62 mg/g dm. In comparison with medicinal plants from China [23] and Europe [15], the investigated African plants were richer in phenolic acids, because their contents were about five times higher. These differences may be due to the fact that they represented different botanical plant species grown in various climate zones. Climatic conditions such as sunshine intensity, rainfalls, dry periods, floods and other factors can have a strong impact on the biosynthesis of phenolic acids [23].

3.2.2 TPC

The TPC mean level was found to be 85.36 mg/g dm in all samples (Table 4). It is possible to indicate the plant materials with the highest TPC contents. Among them are the leaves of *A. cordifolia*, where the range of TPC was from 137.11 to 154.57 mg/g dm, and the roots of *G. huillensis* with the TPC from 137.58 to 142.33 mg/g dm. Comparing these results with those obtained for Chinese plants used in medicine [23], it can be concluded that African plants have higher TPC.

3.2.3 TFC

As shown in Table 4, the total flavonoid concentration in the investigated plants from Congo was determined to be in the range from 0.27 to 3.77 mg/g dm, and the mean TFC was 1.22 mg/g dm. The highest TFC was found in the sample of *A. cordifolia*, 3.16 mg/g dm on average, and the lowest in the root of *Q. africana*, 0.29 mg/g dm. Similar TFCs were determined in the plants from Central Africa [24] and China [23].

3.2.4 Ascorbic acid content

The mean level of ascorbic acid was found as 33.58 mg/g dm. Its content in all investigated African samples was very different, and it was in the range from 0.48 to 111.63 mg/g dm, as shown in Table 4. The highest content of ascorbic acid was detected in the sample of *P. johimbe* and the lowest in *Q. africana*, on average 70.73 and 0.45 mg/g dm, respectively. This differentiation may be caused by the fact that investigated African plant materials were mainly roots and barks, so the level of ascorbic acid can vary depending on the structure of samples. Quite similar values of ascorbic acid content were found in the studies on European plants [15].

3.2.5 Antioxidant activity

Antioxidant activity determined by three methods is presented in Table 4. The mean antioxidant activity determined by the FRAP method in the studied plant materials from Congo was 6.65 mmol Fe^{2+}/g dm. The highest antioxidant potential was observed in the leaf of *A. cordifolia*, 19.73 mmol Fe^{2+}/g dm, also in the sample of *P. macrophylla*, 11.34 mmol Fe^{2+}/g dm, and in the bark of *S. brazzavillense*, 11508.24 μmol Fe^{2+}/g dm. The lowest antioxidant activity was noticed for the root of *Q. africana*. These results were confirmed by other methods, namely, DPPH and ABTS. When compared with results with those

Table 4: Results of phenolic compounds content and antioxidant activity determination in 10 medicinal plants from Congo

	Arithmetic mean ± SD	Median	Range
Total phenolic acid content (TPAC)*	29.87 ± 27.02	20.24	1.53–83.65
Total phenolic content (TPC) *	85.36 ± 59.25	98.47	3.97–154.56
Total flavonoid content (TFC)*	1.22 ± 0.98	0.82	0.27–3.77
L(+) – ascorbic acid content*	33.58 ± 35.59	18.91	0.48–111.63
FRAP**	664.46 ± 601.50	4663.93	456.12–23875.80
DPPH**	304.50 ± 23.60	251.53	19.33–822.45
ABTS*	147.32 ± 10.51	145.99	11.32–367.72

The arithmetic mean [mg/g dm] ± SD is given (n = 3).
*mg/g dm.; **μmol/g dm.

obtained for the extracts of dog rose [15], it is possible to notice that the antioxidant activity measured by the FRAP method was higher for African plants. On the other hand, the antioxidant activity obtained by the ABTS method for plants from Central Africa [24] was much lower than the values determined for medicinal plants from Congo.

3.3 PCA

PCA was performed in order to find factors responsible for the differentiation of the results of the chemical composition of Congo plants. To construct the experimental database, all results for metallic and non-metallic elements were taken into consideration. The same was done for the results of TPAC, TPC, TFC, ascorbic acid, and antioxidant activity assayed by FRAP, DPPH, and ABTS methods. In this way, the experimental database was obtained with the dimensions of 20 × 10. PCA calculations revealed that the first three principal components described together 77% of the variability among the investigated samples. The eigenvalues of PC1, PC2 and PC3 were 8.0, 4.8, and 2.7, respectively. Figure 1 shows the distribution of the studied African medicinal plants samples in three-dimensional plots PC1, PC2, and PC3. There are several characteristic plant samples in this plot. For example, in the right part of Figure 1, one can see sample No. 2 (*A. cordifolia*), and sample No. 6 (*M. stipulosa*), which is located in front of the plot in the left area. Their location is characteristic, since they are far away from the others, which is caused by specific values of elements and other parameters determined. On the other hand, sample No. 10 (*G. huillensis*) can be found in the central area of the plot, and it is characterized by a low value of PC3. To explain the loading values for particular principal components, it is necessary to study Figure 2. It is clear that PC1 is correlated with several element concentrations, such as Na (positively), Cu, Fe, and Al (negatively), as well as TPC, ascorbic acid, and antioxidant activity. On the other hand, PC2 is negatively correlated with the Mn level in the studied plant samples, and PC3 is negatively correlated with the TPACs. As it was found in the recent application of PCA in the interpretation of experimental data for Chinese medicinal plants [23], this statistical method is also well suited for complex evaluation of results of Congo medicinal plants and finding the factors responsible for the differentiation of investigated materials.

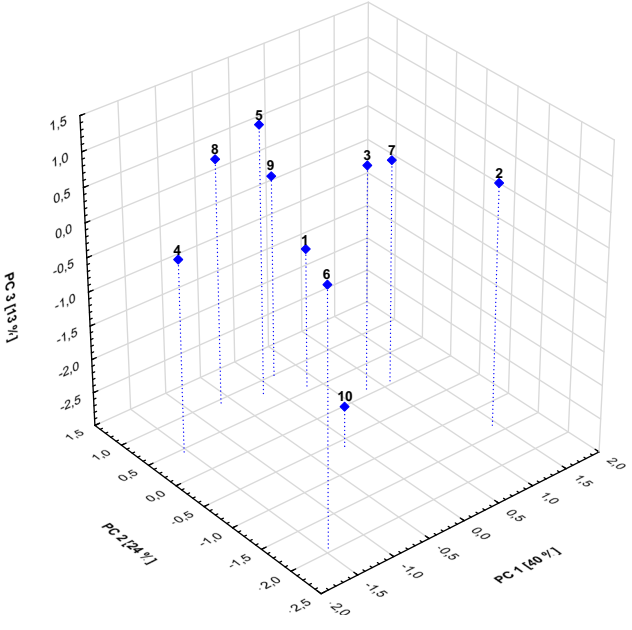

Figure 1: Distribution of the studied African medicinal plants samples in 3D plot of PC1, PC2, and PC3.

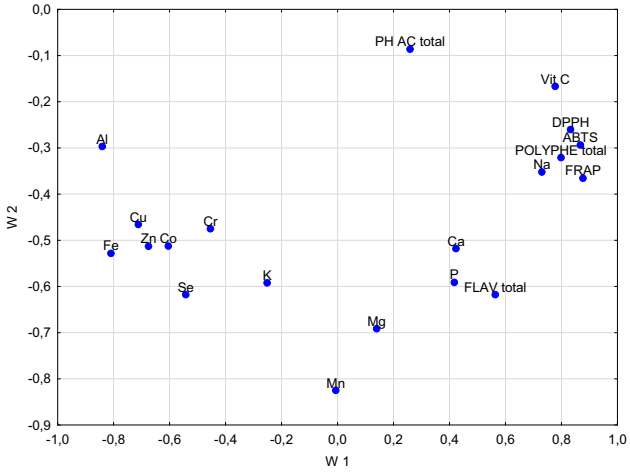

Figure 2: Loading plot obtained for PCA results of African medicinal plants.

4 Conclusions

The research on ten folk medicines used in Congo has shown that it was possible to classify them based on the concentration of elements, phenolic compounds, and ascorbic acid and antioxidant activity. The use of PCA demonstrated that the levels of Na, Cu, Fe, Al, Mn, and also phenolic compound content together with antioxidant activity had the highest impact on differentiation of studied samples. Moreover, a correlation was found between the type of medicinal plant raw material and the concentration of investigated elements.

Particularly this effect was noticed for the samples of *M. stipulosa* and *A. cordifolia*, characterized by a different composition from those of the others, as proved by PCA results. Moreover, the investigated plants do not pose a risk to health; however, the level of harmful elements in folk medicines from Congo should be permanently monitored.

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Informed consent: Informed consent was obtained from all individual participants for whom identifying information was included in this manuscript.

Ethical approval: The conducted research was not related to either human or animal use.

Data availability statement: All experimental data are available in the Supplementary materials for this publication.

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