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Comparison of aqueous and enzymatic extraction combination with sequential filtration for the profiling of selected trace elements in medicinal plants from Kenya

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ABSTRACT

This work presents results for the profiling of eight essential elements (Co, Cu, Ni, V, Mo, Mn, Zn and Cr) in aqueous and enzymatic extracts of eight anti-diabetic medicinal plants, used by Traditional Medicine Practitioners from Nyamira County, Kenya determined by ICP-MS. The plants used in the study were *Solanum indicum*, *Plectranthus barbatus*, *Ultrica dioica*, *Bidens pilosa*, *Solanum mauense*, *Clerodendrum myricoides*, *Carissa edulis* and *Aloe vera*. A sequential filtration procedure was applied to fractionate the elemental contents of the obtained aqueous extracts into molecular size fractions. The results indicate that the low molecular size species (< 3 kDa) were predominant for Mo, Zn, Ni, Co, Mn and Cu, while the moderately large species (10 kDa–0.45 μm) of V were predominant in most of the medicinal plant extracts. In addition enzymatic extraction was compared to aqueous extraction to study the effect of the gastric and intestinal conditions on the release of selected elements from the plants. The amount of the elements extracted by the gastric phase enzymes was higher than the amount extracted by the intestinal phase enzymes. In general, the determined elemental amounts of enzymatic extractions were higher than those of corresponding water extractions for 70% of the elements studied.

1. Introduction

Increasing number of research evidence indicates a positive supporting role of traditional medicine in the management of metabolic disorders such as diabetes, heart diseases and certain types of cancers [1–11]. Each medicinal plant species contains complexes, often unique in nutrients and pharmacologically important phytochemicals including trace elements [11–16,18]. The pharmacological effect of trace elements is in many cases improved by complexation or chelation with ligands, which facilitate their uptake from gastro-intestinal tract via absorption. Thus impairment caused by deficiency of the respective element can be prevented or cured by taking suitable medicinal plants [10]. Pharmaceutically or nutritionally active constituents of medicinal plants are metabolic products of plant cells and also a number of trace elements play an important role [11].

Many studies on the medicinal plants have focused on the isolation and separation of the natural molecular moieties contained in them with the focus of testing their bioactivity. Following established isolation and purification protocols such an approach typically results only in identifying organic compounds and overlooking inorganic species contained therein. Most of the isolated and purified natural products from the plant extracts lose the potency in pure form. This means the natural products and the organic constituents in the plants function in synergy. The levels of trace elements in the medicinal plants should be elucidated. There are only few studies that have investigated detailed elemental composition in the medicinal plants specifically reporting on the levels of potentially therapeutically relevant elements such as vanadium, cobalt, nickel, selenium and molybdenum. Mogwasi and co workers reported on the total levels of trace elements in 19 medicinal plants from Nyamira County, Kenya [3] and further on the potential release of Cr, Mn, Cu and Zn from 12 anti-diabetic medicinal plants [5]. Oyaro et al. analyzedthe metal contents in six medicinal plants from Narok County, Kenya and reported that their infusions had low relative solubility of Co which was safe for application [17]. Maiga et al. reported the levels of nickel in the medicinal and edible plants from Mali to range from 0.7 to 5.2 μg/g [19].

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Determination of total elemental contents in medicinal plants provides a first overview and estimation of potential uptake during treatment of patients or during the use of the plants as nutritional supplements. Most important, total contents are a rather simple check for the presence of the potentially essential or toxic elements. However, meaningful assessment of the pharmacological or nutritional contribution of (trace) elements from medicinal plants requires more detailed investigation of their binding forms and availability. The traditional preparations of plants for medication in many cases use water extraction. Therefore, the determination of the extractable fraction of relevant elements and further characterization of their binding forms is required. For example, Mogwasi *et al.* reported that a substantial amount of Cr, Cu, Mn and Zn in the investigated medicinal plants from Kenya was in the potentially bio-available form [5]. That study applied a sequential extraction scheme to characterize water soluble, oxidisable and reducible fractions. Nischwitz et al. proposed another approach by size fractionation of elemental species in aqueous plant extracts using sequential filtration. This initial study was on two plants and indicated that more than 50% of the extracted Co and Ni were present as low molecular /elemental mass species (< 3 kDa) while vanadium existed mainly as large molecular size fraction (> 3 kDa) in *Bidens pilosa* and *Tabernaemontana stapfiana* from Kenya [9].

A further option to estimate the release of elements from medicinal plants during ingestion is the use of physiologically based extraction protocols simulating gastro-intestinal processes via enzymes. The potentially bio-available form of trace elements can be released by enzymatic action for example of proteins leading to increased uptake in the body. The in vivo studies on the medicinal plant uptake often provide information that cannot be easily interpreted due to physiological discrepancies between humans and the experimental animals used. This has led to the development of in vitro systems based on gastrointestinal extractions which mimic what takes place in a human digestion system. This approach evaluates the fraction of the total metal content in the plant which is solubilised under the simulated conditions thereby becoming available for absorption [20–22]. In vitro experiments are simpler, faster, cheaper, better reproducible and do not raise ethical concerns hence suitable for fast screening of medicinal plants. These processes can only assess the bio-accessibility of trace elements defined as the fraction of the elements dissolved during the digestion process and available for absorption into the circulatory system. The simulated parameters of the human digestive system include stomach and intestinal pH and chemistry. More specifically, the procedures involve simulated gastric extraction with pepsin and extraction in the intestinal stage via a mixture of pancreatin, amylase and bile salts [22]. As the bio-accessibility data is related to the amount of the element taken up into the human blood stream, such data have the potential to derive the exact dosage of the medicinal plants required for therapy.

Based on our previous work the current study investigates the molecular/elemental size fractionation of species in aqueous extracts of medicinal plants from Kenya in more detail and compares the results with an enzymatic extraction protocol. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used for determination of selected trace element profiles in the extracts. Size fractionation of elemental species in the plant extracts was performed by sequential filtration including ultra filtration [9]. In parallel, physiologically based extraction was applied following a published procedure [22]. The study focused on aqueous extracts because various medicinal plant species are used either in the form of water extracts or decoction by the local people in different regions of Nyamira County, Kenya. A set of 8 medicinal plants which were reported in the previous work [5] to have high levels of V, Co, Cr and Ni was selected and the size fractions of elemental species, bio-available and bio-accessible forms of selected essential or potentially essential elements (Co, Cu, Ni, V, Mo, Mn, Zn and Cr) were investigated in this study.

2. Experimental procedures

2.1. Recruitment of herbalists and sampling of plant materials

Details on the recruitment of Traditional Medicine Practitioners (TMPs) practicing in Manga sub county of Nyamira, Kenya were described in previous work [3,5,9]. Based on the information obtained from the TMPs the most commonly used plants and their protocols used for preparation were established. Ten TMPs from Manga sub-county were recruited and requested to provide one kilogram of the dry plant sample of each plant species. The plant materials were collected from the TMPs between February 2014 and June 2014. The plant samples collected were botanically identified, washed with deionised water to remove soil and other adsorbed material, placed in paper bags, sealed, air-dried under shade and ground using a wooden pestle and mortar (for all the samples) to avoid contamination.

This was done with ultimate care to avoid contamination during collection and storage of the medicinal plants. Each sample was placed in a labeled paper bag and stored in a cool dry room until analysis. The following medicinal plants were identified for this study: *Solanum indicum* (S i), *Plectranthus barbatus* (P b), *Ultrica dioica* (U d), *Bidens pilosa* (B p), *Solanum mauense* (S m), *Clerodendrum myricoides* (C m), *Carissa edulis* (C e) and *Aloe vera* (A v).

2.2. Reagents, chemicals and apparatus

All the reagents used in this study were of analytical grade. Calibration standards were prepared from Certipur stock solutions (Merck, Darmstadt, Germany) in 3% nitric acid. De-ionised water was prepared using a Millipore system. Reagents for enzymatic digestion were pepsin, sodium malate, sodium citrate, lactic acid, acetic acid, bile salts and pancreatin.

2.3. Determination of elemental contents by ICP-MS

For quality control the total elemental contents were determined by closed vessel microwave digestion with subsequent analysis using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Prior to microwave digestion additional fine grinding of the medicinal plant material was performed using a ball mill of zirconium oxide vessels and balls (pulverisette 6, Fritsch, Germany). Aliquots of approximately 50 mg of the ground plant samples were digested in duplicate using 2 mL of nitric acid (suprapur, Merck, Germany) and 1 mL of hydrogen peroxide (suprapur, Merck, Germany) in a MARS 5 closed vessel microwave system (CEM, Germany) at 160 °C. Complete digestion of the organic matrix was achieved with occasional presence of silicate residues, which were acceptable for the purpose of this study. The digestion solution was transferred to calibrated polystyrene sample vials and made up to 10 mL with de-ionised water. Blanks and plant reference materials were processed in the same way. The levels of Co, Cu, Ni, V, Mo, Mn, Zn and Cr were analyzed in the sample digests using an ICP-MS Agilent 7500 quadrupole ICP-MS with collision cellin He-Mode (Agilent Technologies, Japan). A micromist nebulizer with double pass spray chamber was applied. The He-flow rate of the collision cell was 4 mL /min, the sample uptake rate was 400 µL/min using an argon low rate (nebulizer gas) of 0.98 L/min. The mean and standard deviation from replicate digestion and measurement were calculated (n = 2) (the ICP-MS software determined the amount of each element in each sample in triplicates i.e. six readings for each element were determined for replicate dilutions of each digestion). NIST 1547 peach plant reference material bought from LGC standards, Germany, was analyzed for quality control.

2.4. Size fractionation of elemental species in aqueous extracts of medicinal plants

140 mg of ground medicinal plant material were mixed with 40 mL de-ionised water in a polypropylene tube and shaken for 13 h in the dark on a horizontal shaker at 100 motions per minute(n = 2)at room temperature. The obtained extracts were first filtered using a 5 µm syringe filter. An aliquot of15 ml of the obtained filtrates was then filtered through 0.45 µm syringe filters. An aliquot of 8 ml of the second filtrate was subjected to ultra filtration through 10 kDa membrane using Amicon filtration units at a speed of 4000 g (Merck-Millipore, Germany). An aliquot of4ml of the third filtrate was finally subjected to ultra filtration through 3 kDa membrane using Amicon filtration units at a speed of 4000 g (Merck-Millipore, Germany). The ultra filtration units were pre-cleaned by filtration of 0.5% nitric acid and deionised water prior to filtration of the samples following previous work [9]. The water extracts and filtrates obtained from sequential filtration were analysed by ICP-MS in He-Mode using external calibration with Rh as the internal standard. NIST 1640a natural water reference material and NIST 1547 plant reference material were analyzed for quality control.

2.5. Enzymatic extraction of elements from medicinal plants

The in vitro gastrointestinal digestion method used was based on Intawongse and Dean [22]. An aliquot of medicinal plant material of 0.3 g was placed into a 50 mL polypropylene tube and treated with 30 mL of gastric solution (1.25 g of pepsin, 0.5 g of sodium malate, 0.5 g of sodium citrate, 420 µL lactic acid and 500 µL acetic acid, made up to 1 L with de-ionised water and the pH adjusted to 2.5 with hydrochloric acid). The mixture was shaken at 100 rpm in an incubator maintained at 37 °C for one hour. The solution was centrifuged at 3000 rpm for 10 min and then5 mL aliquot was removed and filtered through 0.45 µm syringe filter. The aliquot was replaced by 5 mL of the original gastric solution to retain the original solid to solution ratio in the extraction tube.

Subsequently, in order to simulate the small intestine digestion conditions, 52.5 mg bile salts and 15 mg of pancreatin were added into each extraction tube and saturated sodium bicarbonate solution was added to adjust the pH to 7.0. The mixture was then shaken at 100 rpm in an incubator maintained at 37 °C for 2 h when a second 5 mL aliquot was removed and filtered. The final sample was used to check that small intestinal equilibrium had been attained. All aliquots were stored at 4 °C and analyzed by ICP-MS at 20-fold dilution for the gastric extracts and 30-fold dilution for the intestinal extracts to minimize matrix effects. For comparison aqueous extracts containing only de-ionized water as extractant were processed in the same way (same incubation time and temperature) in parallel and analyzed at 5-fold dilution. In all cases blanks were processed in analogy to the samples for correction.

The topping up of the remaining extract of step 1 (25 mL) to 30 mL and addition of solid reagents for step 2 requires correction of the determined elemental amounts in step 2 to avoid duplication of results from step 1.For this purpose the absolute extracted amounts of the element of interest in step 2 was calculated by multiplying the concentration in the final extract with the volume (30 mL) and subtracting the concentration determined in step 1 multiplied by the remaining volume of 25 mL. Due to these difficulties, the elevated blank levels of some reagents and the uncertainties associated with separation of the solid residue from the supernatant, drying, aliquoting and digestion of the residue, the determination of the residual fraction for mass balance purposes was not performed.

3. Results and discussions

3.1. Size fractionation of extracted elemental species

Aqueous extracts were performed in duplicate for 8 selected medicinal plants used for the management of diabetes by TMPs. A sequential filtration approach previously tested for only two medicinal plant extracts [9] was applied to study the molecular size distribution of relevant elements in the extracts. Elemental amounts in the size fractions, that means the concentration or percentage of an element in a specific particle size or molecular size obtained by sequential filtration, < 3 kDa, 3 kDa – 10 kDa, 10 kDa- 0.45 µm and 0.45 µm–5 µm determined by ICP-MS are summarized in Table S1 (Complementary Information).The percentage extraction efficiency was calculated as ratio of the elemental concentrations in the aqueous extract (5 µm filtrate) and the total element concentrations obtained from microwave digestion (Table S1). The size fractionation for V, Mn and Zn is presented in Figs. 1–3. The respective figures for the other elements are shown in the Supplementary Information (Figs. S1–S4). Data for Cr is not shown due to very low concentrations and potential interference problems in ICP-MS determination leading to negative results for most size fractions.

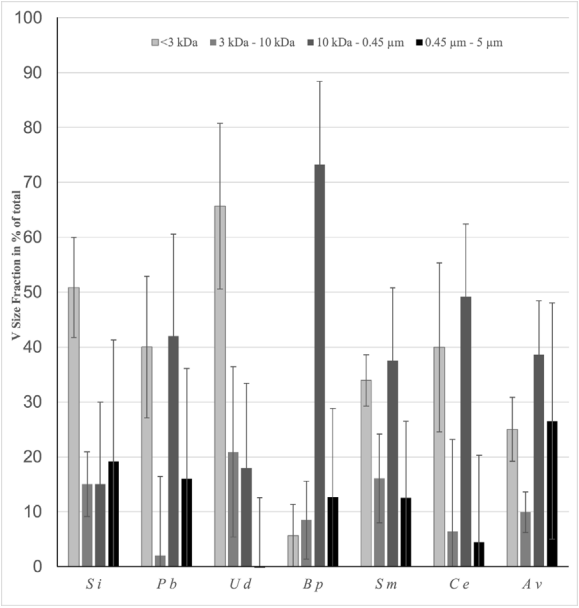

Fig. 1. Percentage distribution of V across size fractions obtained from sequential filtration of aqueous extracts of selected medicinal plants.

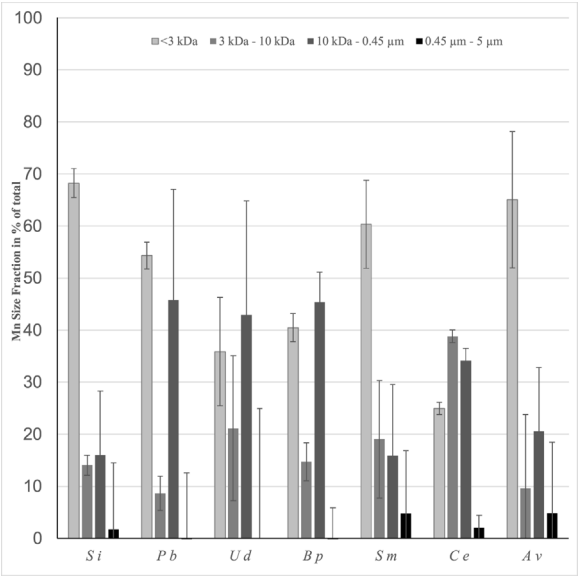

Fig. 2. Percentage distribution of Mn across size fractions obtained from sequential filtration of aqueous extracts of selected medicinal plants.

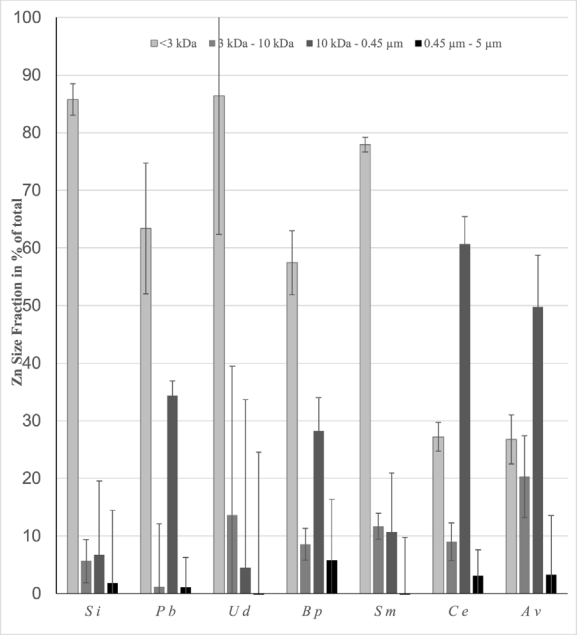

Fig. 3. Percentage distribution of Zn across size fractions obtained from sequential filtration of aqueous extracts of selected medicinal plants.

ICP-MS results were backed up by analysis of water reference material NIST 1640a with recoveries in the range of 96% to 104% for Co, Cu, Ni, V, Mo, Mn, Zn and Cr.

The determination of the extracted elemental contents and their molecular species is vital for the understanding of the molecular mechanism of the potential therapeutic properties or toxicity of the plant derived elemental species. Sequential (ultra)filtration with offline detection is an appropriate technique for size fractionation of elemental species as it maintains species stability and exhibits good recovery percentage. This allows a rapid screening of the size distribution of the elemental species. Detailed molecular identification and structural characterization requires much higher effort in such complex plant extracts. The elemental analysis showed that the mass distribution across these fractions in the medicinal plants was not the same.

Vanadium in most medicinal plants occurred abundantly in the 10 kDa - 0.45 µm size fraction except for *S i* and *U d* in which the low molecular weight fraction of < 3 kDa was predominant (Fig. 1). For the latter two plants only 34% and 13%, respectively, of total extracted amount of V was found to be in the high molecular weight fractions > 10 kDa. In the remaining medicinal plants 50%–86% of vanadium was in the weight fractions of > 10 kDa. This confirms our previous study [9] indicating that a substantial amount of vanadium is present in high molecular weight fractions. This is likely to have a major influence on vanadium uptake via oral application of the plant extracts and may turn out to be beneficial compared to the variety of synthesized low molecular weight vanadium insulin mimetic drug candidates.

For the other investigated elements the percentage of high molecular weight binding forms (> 10 kDa) in all plants is below 50% with exception of the plants *C e* and *A v* for Co and Zn, the plant *C e* for Cu and the plant *B p* for Mo. This indicates that the large percentage amount of vanadium in the high molecular weight fractions is not a general effect observed for many elements and is not due to clogging of the filter membranes.

Manganese predominantly exists as low molecular weight species (< 3 kDa) in the extracts of plants *S i*, *S m* and *A v* (> 60%). In the remaining investigated plants the size fraction of 10 kDa–0.45 µm reaches up to 45% and is in some cases the most abundant fraction (Fig. 2). This demonstrates that for different plant species Mn can be present in rather different size fractions. However, the fraction 0.45 µm – 5 µm accounts for < 5% of Mn in all plants.

Zinc predominantly (up to 85%) existed as low molecular weight species (< 3 kDa) in the medicinal plant extracts except for *C e* and *A v*, where the high moderate molecular weight species (10 kDa–0.45 µm) were most abundant (Fig. 3). In those two plants 64% and 53%, respectively, of total Zn in the extracts were present in high size/molecular weight binding forms (> 10 kDa). Highest concentration of zinc of 25 mg/kg was found in < 3 kDa size fraction from *S m* while the lowest of 2 mg/kg was in *C e*. High molecular weight species in size fraction of 0.45 µm-5 µmwere detected only in low amounts (< 6%).

The distribution of Co and Cu across the obtained size fractions is very similar to that of Mn and Zn. Also in this case they occur predominantly as low molecular weight species (< 3 kDa) in plants *S i*, *U d*, *S m*, while the 10 kDa-0.45 µm molecular weight species was the most abundant in *Av* and *C e* (Supplementary data Figs. S1 and S2).

Nickel existed abundantly in the low molecular weight fraction (< 3 kDa) in all the medicinal plants with 57%–93% of total extracted amounts (Supplementary data Fig. S3).

Molybdenum was found nearly exclusively in the fraction < 3 kDa in the extracts of *Ud* and *Av* (Supplementary data Fig. S4). However, in *B p* more than 80% of extracted Mo was detected in the 10 kDa–0.45 µm fraction.

These results are similar to those reported by Nischwitz et al. for two medicinal plants from Nyamira, Kenya using hot water extraction of V, Cr, Mn, Zn, Cu, Co and Ni [9] but are different from those reported by Rodolf et al. on patterns of Co, Mo and Ni in fungi porcini (*Boletus-sedulis*) mushroom [23].

Mn and Zn in most plants existed predominantly in molecular size of < 3 kDa but V species predominantly existed in this size only in *S i* and *U d*. This finding is in agreement to the numerous studies focusing on the synthesis of low molecular weight insulin-mimetic drugs for oral treatment of diabetes. The plants contain essential elemental species which are relevant in the management of diabetes. However, in the case of vanadium, 5 out of the 7 investigated medicinal plants contain > 50% of extracted vanadium compounds in the fraction > 10 kDa. Preparative isolation of these high molecular /elemental weight fractions would be relevant to compare their uptake and insulin mimetic properties with synthetic low molecular mass drugs. The sequential filtration of the plant extracts provides a rapid overview on the most abundant size fraction of the elements indicating significant differences between the medicinal plants. Further effort is required to characterize and identify the elemental species in these fractions. In addition, the fractions should be tested on cell assays for their insulin mimetic effects to direct the efforts of speciation analysis on the efficacious medicinal plant extract fraction. Moreover, information on uptake of the elemental species after ingestion is required to assess further their bioaccessibility and potential therapeutic relevance.

3.2. Bio-accessibility of trace elements from the medicinal plants

A published protocol for 2-step enzymatic extraction was followed. For comparison water extracts were performed in parallel with same incubation time and temperature to evaluate whether the enzymatic procedure increases the extracted amount of the monitored elements (Figs. 5 and 6). When applying the protocol from Intawongse [22] two challenges were encountered: First, blank levels in some of the reagents were rather high and thus prevented accurate determination of low extracted amounts of some elements. In particular there was interference in the gastric phase extracts due to high levels of V, Cr, Cu, Zn and Mo in one of the reagents (pepsin) resulting in values below limit of detection after blank subtraction for some of the plants (Fig. 4). Some of the enzymatic reagents could be contaminated with these elements and need to be purified for future studies as the manufacturers intended them to be used for biochemical studies involving organic components. In case of Mn the extracted amounts were still much higher than the

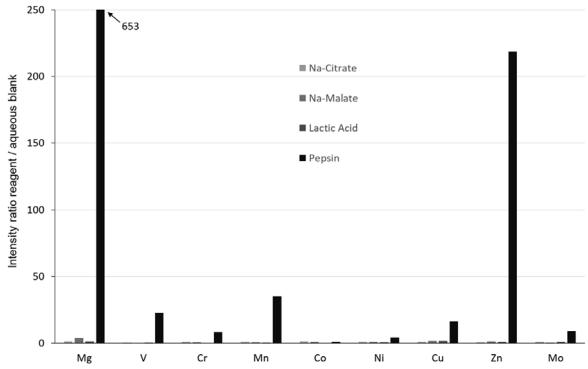

Fig. 4. Blank levels in the reagents used for enzymatic extraction shown as intensity ratio relative to the aqueous blank.

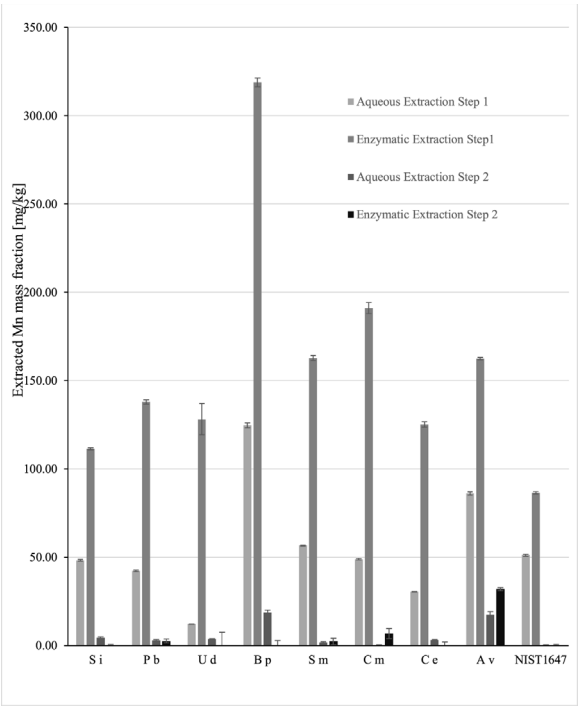

Fig. 5. Comparison of 2-step aqueous and enzymatic extraction for Mn in selected medicinal plants.

elevated blank levels. Second, due to the addition of the solid reagents of the second step to the remaining extract of the first step, the amount extracted in the first step needs to be subtracted from the extracted amount in the second step to avoid duplication of the results (see experimental section).

Comparisons of aqueous and enzymatic extraction as a first step to characterize the elemental species present in plants are shown in Figs. 5 and 6 as well as in the Supplementary Information (Figs. S5–S10). For Mn, Co, Zn and partly for V there is an increase of the enzymatically extracted mass fraction in step 1 compared to the aqueous extraction (Table S1). In the second enzymatic extraction step the released elemental mass fractions are lower than in the first step with few exceptions. Increased extractions in the enzymatic step 2 are observed for Co and Cu in most investigated plants and in single or few plants for the other elements (Table S1).

This shows that the in vitro enzymatic release of hypoglycemic trace elements is higher than that released from the aqueous conditions. The increase is clearly demonstrated for 60% of the investigated medicinal plants for Mn and Zn (Figs. 5 and 6). However, the percentage increase due to enzymatic activity for the same element varies in quite a large

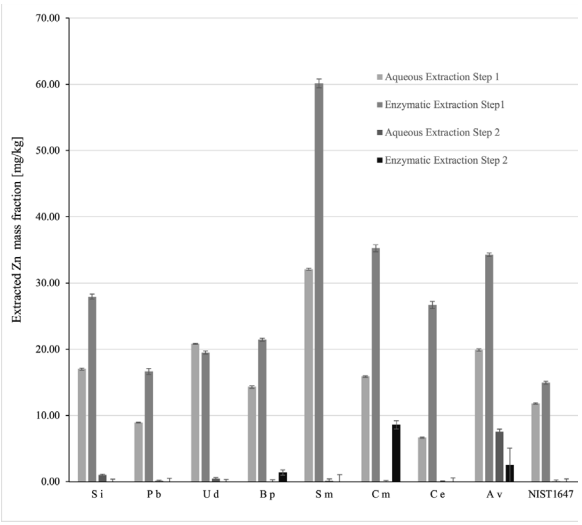

Fig. 6. Comparison of 2-step aqueous and enzymatic extraction for Zn in selected medicinal plants.

range for the specific medicinal plant. For example, Mn extraction increases by 90–950% indicating the different composition of medicinal plants and their different binding properties of Mn species. The enzymatic extraction in Step 1 increased the extracted amount of Co by 58% compared to the water while in Step 2 the increase was230% in comparison to water. The increase in the extraction efficiency for cobalt due to the gastric phase mimicking enzymes ranged from 11% (*Bidens pilosa*) to 170% (*Ultrica dioica*). The highest increase in the intestinal enzymatic phase mimicked physiologically extraction of cobalt was 530% for *B. pilosa* while there was a decrease by 73% in *S. indicum*. Similar extraction trends were observed for Zn, Cr, V, Ni and Cu but with different extraction percentages in each step and in each medicinal plant species. The extraction of the investigated elements was generally favored in neutral intestinal medium while extraction in acidic gastric phase resulted typically in lower extracted amounts with few exceptions, for example for Cr in some samples.

We have also included a plant reference material (NIST 1547, Peach leaves) in this experiment to enable comparison of our results with other studies focusing on similar enzymatic protocols. The quality and activity of the enzymes is critical for such physiologically based approaches and may lead to significant variation between different studies. Thus the results for a well-known reference material can help to normalize the results obtained from different enzyme batches.

Appropriate dilution of the enzymatic extracts was performed to minimize matrix effects during the measurement using the same external calibration with Rh as the internal standard. Arika et al. reported the total levels of vanadium in Kenyan medicinal plants used by TMPs to manage diabetes at a range of < 0.2 to 17.6 mg/kg [25]. Raju *et al.* reported the leaves of *Catharanthus roseus* to contain the least concentration of vanadium of 2.1 mg/kg while the roots of *Pinax ginseng* had the highest concentration of 106 mg/kg among medicinal plants used for cancer management by TMPs [11]. The dosage of pinax ginseng prescribed daily by the TMPs for cancer management was found to be about300 mg, which approximates to total vanadium content of about 0.03 mg. Vanadium based drugs were found to be effective against different cancer cells taken directly from patients. Vanadium exhibited some unique beneficial effects to its anti-carcinogenic potential under very low dosage without any adverse toxicity [13]. Many vanadium compounds have special therapeutic properties, which are being used in the treatment of diabetes [3,9]. A study done by Thompson et al. reported that bis(ethylmaltolato)oxovanadium(IV) (BEOV) and bis(maltolato)oxovanadium (IV) (BMOV) have potential to manage *diabetes mellitus* [24]. The main drawback of initially applied

inorganic vanadium species is the low resorption after oral application. This was improved by the use of low molecular weight organo-vanadium complexes. For *Solanum indicum* and *Clerodendrum myricoides* with higher vanadium contents than the other investigated plants there is a clear increase of the extraction efficiency of the enzymatic phase 1 compared to the aqueous extraction (Fig. S5). However, for most of the other plants the vanadium blank in the enzymes is too high for detection of the low amount of extracted vanadium.

The bio-accessibility of Mn, Mo, V, Cr, Zn, Co and Ni in the medicinal plants decreased during the transition from the gastric phase to the intestinal phase. Similar results were reported in previous studies [21,26,27]. This could be due to the fact that bio-accessibility of these elements correlates negatively with pH. The result for Cu differs greatly between different matrices and extraction phases as reported by Intawongse & Dean and Turner & Hambling [22,28]. These findings are similar to those of Navorro et al. who reported that the levels of trace elements extracted in the gastric phase was more than that of the intestinal phase in mussel tissue [20], however, they are in contrast to those reported by Intawongse et al. which showed that the intestinal phase extracts contained higher amounts of trace elements in vegetables grown in contaminated soils [22]. This means that the amount of the trace elements bio-available to the human body is larger than that which is extracted by water, thereby proving that enzymes are more efficient in the mobility of species [5].

The large number of diabetic patients globally requires more effort in the development of therapies to substitute insulin for the control of the blood sugar levels. Cr, Mn, Zn, Mg and V species have been reported to be hypoglycemic. Kenyan medicinal plants used for management of diabetes by TMPs such as B. *pilosa*, *C. edulis*, *E. abyssinica*, *A. pluriseta* and *S.henningsii* have been reported by Ngugi and co workers to lower blood sugar levels in aloxan-induced rats [29] and other researchers have reported that *B. pilosa*, *C. edulis*, *E.abyssinica* contain high levels of hypoglycemic elements [5,25,29]. The aqueous extracts of the eight investigated medicinal plants were found to contain hypoglycemic elements and their therapeutic potential may be attributed to the presence of V, Cr and Mn.

4. Conclusion

All the medicinal plants investigated were found to contain vanadium, chromium, cobalt, nickel, molybdenum, manganese, zinc and copper in different size fractions and species. The elemental distribution across the fractions obtained from sequential filtration varies in size among the medicinal plants. The sequential filtration technique determines the water extractable molecular size species of the elements which are potentially involved in the hypoglycemic therapeutic action of the investigated plants.

The elemental extraction results show that all the medicinal plants contain elements vital in human metabolism that are required for growth, prevention and treatment of various diseases. The eight plants; *S.indicum*, *P.barbatus, U.dioica, B.spilosa*, *S.mauense, C.myricoides, C. edulis,* and *A.vera* may be used for the management of *diabetes mellitus* as they contained substantial bio-accessible amounts of Mn, Cr, V and Zn, which have been shown to be hypoglycemic.

The enzymatic protocol as adopted from the literature seemed to be a more efficient extraction technique as more of the elements were extracted in most plants than that extracted b compared to extraction with water. Though the blank levels for some elements were quite high and this poses a problem in the analysis of elements which are in low concentration in the plants. This is in particular the gastric phase which had substantial blank levels for vanadium (Fig. 4). This can be overcome by proper purification of the enzymes.

It is proposed that the medicinal plants need more pharmacological studies to establish the organic ligands of Mo, Zn, Cr, V, Ni, Mn and Co in the medicinal plants in order to elucidate the organometallic and metalloprotein compounds in the medicinal plants using hyphenated techniques like HPLC-electrospray (ESI)-MS/MS and HPLC-ICP-MS. However, in many cases it will hardly be possible to determine the exact molecular structure and metal binding form. Therefore, the size fractions obtained by sequential filtration can be considered as operationally defined species and tested for their anti-diabetic properties in cell assays to achieve a fast screening and identification of the most therapeutically active fraction of the plant extracts.

Conflict of interest

The authors declare that there is no conflict of interest whatsoever.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jtemb.2019.03.003.

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