Feasibility of ion-exchange solid phase extraction inductively coupled plasma mass spectrometry for discrimination between inorganic As(III) and As(V) in phosphate-rich *in vitro* bioaccessible fractions of *Ayurvedic* formulations [‡]

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Abstract

Determination of potentially toxic elements in the µg/kg - mg/kg concentration range in some *Ayurvedic* formulations was performed after microwave-assisted acid digestion and inductively coupled plasma mass spectrometry. Arsenic and Hg concentration in the mineral formulations reached about 10% and 0.6% by weight, respectively. Arsenic bioaccessibility was not influenced by pepsin and pancreatin containing model suspensions of pH = 1.2 and 6.8 (with 0.05 M K_2HPO_4), respectively. The bioaccessible As fraction of the mineral formulations was about 10-20%, even if the estimated As intake from these products exceeded the 3.0 µg/ BW kg/day benchmark dose limit with an increased incidence of 0.5% for lung cancer ($BMDL_{05}$) caused by inorganic As by about 25 times. Ion-exchange solid phase extraction procedure was applied to discriminate between iAs species in either the initial bioaccessible fractions obtained by incubation in simulated duodenal juice or by spiking them with 75 and 150 µg/L arsenate ions [iAsV]. The complex sample/extractant matrix caused a 60-70% recovery for iAs(V) and a breakthrough of about 20%. Suppression of phosphate ions in the extractant improved iAs(V) recovery by about 10%. Arsenic breakthrough was not observable at the 75 µg/L iAs(V) spike level. Similar results were obtained on a *homeopathic* product confirming the interference of phosphate on As speciation in bioaccessible fractions of complementary and alternative medicines (CAMs). Conversion rate of iAs(III)/iAs(V) in Ayurvedic products with large As content could be estimated. This approach is more informative than extrapolation of results by X-ray based techniques on solid state of CAMs.

1. Introduction

Traditional medicine is a system of treatment transmitted more or less in unchanged form. The WHO defines it as complementary and alternative medicine (CAM). According to WHO, Chinese traditional medicine, *Ayurvedic* or Indian traditional medicine, the Tamil siddha, the Persian – Arabic *unani*, homeopathy, etc belong to this category. Among these, *Ayurveda* is the second most popular after the Chinese traditional medicine. The main difference between these two systems of treatment is that *Ayurvedic* formulations may contain not only medicinal plants but also minerals and elementary state elements (e.g., Hg, sulfur). In the developing world, 70-95% of the population relies on the traditional medicines for primary care because of being less expensive as a result of using the natural resources of the given country [1]. From data collected from 20 European countries, it was extrapolated that 56% of the European population in general had used CAM at least once in 2012 [2]. The extrapolated prevalence of CAM use by children in Europe was 52% [2]. Its increasing popularity is due to side effects and lack of curative treatments for several chronic diseases, high cost of the new medicines, microbial resistance developed against different active ingredients as well as lack of empathy experienced by patients during medical treatments [3-6]. The *Ayurvedic* formulations are classified as food supplements by the 2002/46/EC directive, and consequently they are sold as over-the-counter drugs. The role of the aforementioned directive is not only to enable their free movement in the internal market but also to ensure a high level of protection for consumers and facilitate their choice [7]. However, these food supplements are not subject to the strict clinical studies like pharmaceutical products, therefore they may represent a health risk.

The *Ayurvedic* formulations can be classified according to their preparation procedures, therapeutic use, presentation form (tablets, capsules, syrup and ointment [8]). For analysts, classification based on the ingredients is more useful. According to this

classification, herbal, herbomineral and animal formulations can be distinguished. Among the metals used for the preparation of *Ayurvedic* formulations, Hg has a primordial importance, while among minerals, As-containing ones such as realgar (α-As_4S_4) and orpiment (As_2S_3) are popular additives. *Bhasma* and *sindoor* are two important types of metallic formulations as being often the main component of the given formulation. *Bhasma* is obtained by the joint calcination of metals, minerals with plants after previous purification such as sublimation. *Sindoor* is a powder consisting mainly of Hg(II) sulfide in form of red cinnabar [9]. The increasing popularity for the use of *Ayurvedic* products is reflected by the increasing number of scientific works (i.e., about 9500) published in the last 20 years mainly in the medicinal, pharmacological and toxicological fields, although the largest number of publications has been registered in India [10].

The principle of homeopathy founded in 1796 is based on the introduction of toxic substances into the human organism at a very low concentration because the low dose would have a curing effect. Moreover, the larger the dilution rate of the harmful substance chosen, the more efficient is the fight against the adverse symptoms. Therefore, three dilution methods are generally used in homeopathy: decimal (indicated with D, DH, X), centesimal (C, CH) and fifty thousand-fold (LM). However, the chosen substance is repeatedly diluted in alcohol or distilled water, each time with the containing vessel being struck against an elastic object. Its popularity is based on the presumption that it offers the perfect solution for the chronic diseases where conventional medicine fails. Homeopathic products are worth to be investigated as representing a challenge for an analyst to detect the low concentration of their ingredients. Nevertheless, matrix effects in the aqueous solutions will presumably be lower or inexistent.

It is well-known that elemental toxicity (e.g., As, Hg) depends on oxidation states and chemical environment. Therefore, elemental speciation gives more realistic information.

Conventional As speciation methods involve high performance liquid chromatographic (HPLC) separation on ion-exchange (IE) columns followed by hyphenation to an atomic spectrometric detector most commonly nowadays, inductively coupled plasma mass spectrometry (ICP-MS). Development of alternative methods aiming at reducing the operational costs by substituting either HPLC or ICP-MS, or both has been attempted. Various solid phase extraction (SPE) cartridges enabling on-site separation were investigated for speciation of iAs(III), iAs(V) and methylated forms of As(V). Among them, solid phase sorbents including IE resins [11-15] have been widely employed for As speciation.

Having gained positive experiences with SPE minicartridges for As speciation of drinking [13, 16] and geothermal waters [17], the objective of the present study was to extend our knowledge to speciation for As-containing *Ayurvedic* products characterized by complex sample matrices. Up to now, As speciation in Ayurvedic formulations has been performed by X-ray based analytical techniques directly in the solid state of matter. Thus, our main objective was the development of an IE-SPE-based As speciation method as an alternative for the conventional HPLC-hyphenated techniques in bioaccessible fractions of some As-containing *Ayurvedic* formulations by modelling gastric juice and duodenum fluid. Moreover, determination of other potentially toxic element concentration in these formulations was also aimed by ICP-MS taking advantage of the multielemental capability of this analytical technique.

2. Materials and methods

2.1. Materials and reagents

Throughout the experiments, the deionized water (DW) with a resistivity of 18 MΩ cm was taken from an ELGA Purelab Option-R7 ultra-pure water unit (ELGA LabWater/VWS Ltd.,

High Wycombe, UK). Concentrated HNO_3, 67% by weight and 30% by weight H_2O_2 were purchased from VWR International (Radnor, PA, USA), were of Normatom® and AR® qualities, respectively. For external calibration, 1 g/L of the corresponding acidic elemental stock solutions (Merck, Darmstadt, Germany) were used after appropriate dilutions. Concentration ranges of the calibration solutions were as follows: $0.1 - 5$ µg/L for Hg, $1 - 10$ µg/L for Sn, $10 - 100$ µg/L for Cr, Mn, Ni, Cu, Cd, Ba and Pb; $10 - 1000$ µg/L for Zn; $10 - 1000$ µg/L for Fe and As. For speciation analysis, the iAs(V) stock solution was prepared from solid KH_2AsO_4 purchased from Sigma Aldrich (St. Louis, MI, USA). All standard solutions were prepared daily from stock solutions via appropriate dilutions with DW in PP Falcon® centrifuge tubes (Fisher Scientific, Waltham, MA, USA). The final HNO_3 concentration of each sample solution was set to 5% (v/v) except for determination of Sn. In this latter case, the solutions also contained HCl in 1 % (v/v). Moreover, for Hg determination, the intermediate stock solution of 1 mg/L contained HCl in 20% by weight to prevent Hg loss. Before use, the 50-mL centrifuge tubes were soaked in 20% (v/v) HNO_3 for 24 h and then rinsed with DW.

For mimicking gastric juice, 37% by weight HCl of TraceMetal[TM] purity was used (Fisher Chemical, Loughborough, UK). The high purity NaCl was purchased from Salinen Ltd. (Austria). Finally, pepsin synthesized for proteomics was purchased from VWR International. For mimicking duodenal juice, 0.2 M NaOH solution prepared from 98.0% purity solid reagent (Sigma-Aldrich), solid powder of pancreatin (PanReac AppliChem, Darmstadt, Germany) and high purity KH_2PO_4 (Fisher Scientific) were used. The 0.45 µm pore size Acrodisc® membrane filters applied during the bioaccessibility study were purchased from Sigma Aldrich.

2.2. Study design

In the present study, seven different *Ayurvedic* formulations were purchased from India through one of the major e-commerce sites. One of them, a herbal product (*Swasari Ras*) could even be purchased from two different lots. The keywords (i.e., *talaka/haritala*, *manahsila* and *malla*) with reference to As meaning orpiment, realgar and white arsenic, respectively, were chosen according to [18] and inserted into the search engine. Another *Ayurvedic* capsule formulation containing Asiatic pennywort (*Centella Asiatica*) was purchased directly from one of the biggest CAM stores of Budapest due to previous works reporting relatively high As content of this plant material [19]. Finally, a homeopathic *Acidum Arsenicosum* Anhydricum 9 CH (Boiron, Messimy, France) was purchased from a pharmacy in Budapest for comparison purposes.

Among the *Ayurvedic* formulations, seven corresponded to orally administrated CAM and another one (*Pama Malam*) was an ointment. Two colostrum samples of bovine origin were purchased. Three other products (i.e., *Swasari Ras, Zandu Shwas Kuthar Rasa* and *Pama Malam*) were herbominerals. According to the information leaflet accompanying the delivered products, *Zandu Shwas Kutar Rasa* contained 6.3% by weight of each purified Hg, realgar, borax and sulfur. As plant materials, it contained aconite (*Aconitum ferox)*, dried ginger (*Zingiber officinale*), long pepper (*Piper longum*) and black pepper (*Piper nigrum*) in 6.3%, 6.3%, 6.3% and 49.5% by weight, respectively. In return, the composition (% by weight) of *Pama Malam* was as follows: sulfur (5.0%), As_2S_3 (2.5%), and α-As_4S_4 (1.3%), camphor tree (*Cinnamomun Camphora*) and katechu tree (*Acacia catechu*), the last two both in 2.5%. The *sindoor* formulation contained purified Hg, traces of Hg(I) and Hg(II) chlorides as well as As(III) oxide. Another formulation, *Qurs Kushta Qalai* was sold as an aphrodisiac. According to literature data, the *kushta* included in the present study is a mixture of alum and Sn, added in molten phase to Aloe vera (*Aloe barbadensis*) extract just as the mineral ingredients of *sindoor* [20]. Detailed information on the origin and composition of the

investigated products can be seen in Figure S1 (Appendix) and Table S1 (Appendix) of electronic supplementary material, respectively.

2.3. Apparatus and analytical instrumentation

Solid residues were dried in a MLW WSU 100 electric oven (MLW, Leipzig, Germany). Samples were weighed on an ABJ 220-4NM analytical scale (KERN, Stuttgart, Germany) with a resolution of 0.1 mg. For sample homogenization and simulating the peristaltic movements of the digestive system, an Elmasonic S 40 ultrasonic bath (Elma, Singen, Germany) was used. Thermostation of the samples at 37 °C was achieved in a VWB 12 thermostat (VWR International) with a precision of ± 0.2 °C. The pH of the suspensions was adjusted using an OP-211/2 pH meter (Radelkis, Budapest, Hungary). Finally, samples were centrifuged at 4,500 rpm in a MLW T54 instrument (MLW, Leipzig, Germany).

The MW-assisted digestion was performed in an Ethos Plus 1 equipment (Milestone S.r.l. Sorisole, Italy) equipped with a HPR-1000/10S rotor segment. The total organic carbon (TOC) content of the digested samples was checked by a Multi N/C 2100 Analyzer (Analytik Jena, Germany). Elemental analysis was performed on an Element 2 inductively coupled plasma sector field mass spectrometer (ICP-SF-MS) (Thermo-Fisher Scientific, Germany). Prior to ICP-MS analysis, Ge and In, as internal standards (IS) were added in a concentration of 20 µg/L and 10 µg/L, respectively, to the samples. For each sample, digestion and ICP-MS analysis were performed in triplicates. The energy dispersive X-ray fluorescence (EDXRF) analysis of the solid residues was performed on a benchtop MiniPal 2 (PANalytical, Almelo, The Netherlands) instrument equipped with a 12-port rotor segment, an X-ray anode made of Rh and a Si-PIN high resolution diode detector. For energy calibration, a built-in reference standard made of a Cu-Al alloy was used.

2.4. Sample preparation and analytical procedures

The sample preparation protocol of the *Ayurvedic* formulations and homeopathic anhydrous arsenous acid is presented in Figure 1. However, in the case of the ointment, only EDXRF analysis was performed. Except for the capsule formulation of Asiatic pennywort and ointment, 200-400 mg of powdered samples was homogenized with an agate pestle in a mortar made of the same material. In the case of the Asiatic pennywort, the aforementioned amount was obtained by mixing the content of the appropriate number of capsules.

2.4.1. Microwave (MW)-assisted acid digestion ICP-MS analyses

Prior to the elemental analysis, the samples were subjected to MW-assisted acid digestion. The homogenized samples were transferred into 20-mL quartz microvials. Then, 5 mL of 67% by weight HNO_3 and 1 mL of high purity H_2O_2 of 30% by weight were also added. Each quartz vial loosely closed with its cap was transferred into a PTFE digestion vessel (n = 10). After this step, 2 mL of 30% H_2O_2 and 8 mL of DW were added in the outer space of the quartz vials. The detailed MW-assisted digestion program can be seen in Table S2 (Appendix). After completing digestion and cooling down of the vessels, the digested samples were transferred into PP tubes and properly diluted (5-10,000×) with DW for HNO_3 concentration to be set as 5% by volume. For TOC analysis, 100 µL of the properly diluted digested samples were injected into the equipment heated to 800 °C. The flow rate of the oxygen stream was 160 mL/min. As IS for the determination of As and the rest of the potentially toxic elements Ge and In were used. Their concentration in the samples was set to 20 µg/L. The optimized operating conditions for the ICP-MS measurements and the monitored isotopes are listed in Table S3 (Appendix).

2.4.2. EDXRF analysis of ointment and residues of MW-assisted digestion

The *Ayurvedic* ointment and the residues of the MW-assisted acid digestion were analyzed by EDXRF. For this, samples were dried in an electric oven for 2 h at 105 °C. After this step, samples were cooled down and in a desiccator for 24 h. Finally, the dried samples were weighed and analyzed. Before starting the analysis, the EDXRF instrument was calibrated. Then, samples were placed onto the PP film windows of the sample holders. Analysis was carried out at 20 keV and 450 mA for 10 min.

2.5. Arsenic bioaccessibility study

In the present study, the bioaccessibility protocol described by Giacomino *et al.* [21] was applied. Briefly, for mimicking the gastric juice, 3.5 mL of 37% by weight HCl solution was added to a mixture consisting of 1.0 g of NaCl and 1.6 g of pepsin. Then the suspension was made up to 500 mL with DW to set the pH to 1.2. From this suspension, 25 mL was added to 200 mg of *Malla sindoor* and *Zandu Shwas Kuthar Rasa* each as well as 50 mL to 400 mg of Asiatic pennywort, two different lots of *Swasari Ras* and homeopathic anhydrous arsenous acid. Samples were then incubated for 2 h at 37 °C. For modeling the peristaltic movement of the stomach, suspensions were sonicated for 5 min every half an hour. Finally, samples were centrifuged for 10 min at 4500 rpm. The supernatant was separated from the residue and it was filtered through 0.45 μm pore size membrane filters. The filtered and acidified samples were stored at 4 °C prior to the elemental analysis.

For simulation of the duodenum juice, 3.4 g KH_2PO_4 was dissolved in 125 mL of DW. The resulting solution was mixed with 38.5 mL of 0.2 M NaOH solution. Then, 5.0 g of pancreatin and 250 mL of DW were added. The suspension was made up to 500 mL in order to set the pH to 6.8. Then, the aforementioned bioaccessibility protocol was applied. The procedure applying duodenum fluid simulating conditions were also performed with samples spiked with iAs(V) at the 150 μg/L concentration level.

2.6. Discrimination between iAs species in phosphate-rich environments by off-line IE-SPE-ICP-SF-MS

After completion of the incubation of some *Ayurvedic* formulations with duodenum simulating suspension, 20 mL of the supernatant samples collected after centrifugation were subjected to a previously reported IE-SPE [11]. Briefly, this procedure consists of a series of steps such as conditioning of cartridge containing 0.62 g of resin with 40 mL of 0.5 M HCl, washing with deionized water to set pH=5, loading of 20 mL sample with $1-2$ mL/min and its elution with 5 mL of 0.5 M HNO_3 solution. The strong anion exchange material was a styrene, divinylbenzene and ethylstyrene polymer gel functionalized with the chloride form of quaternized trimethylamine having a capacity of 1.2 meq/mL. Its particle size ranged between 200 and 400 mesh. The SPE fractions were digested by using MW according to the protocol described in Section 2.4.1.. The SPE procedure was repeated with samples spiked with iAs(V) at the 75 and 150 µg/L concentration levels.

In the case of the homeopathic product, 500 mg was dissolved into 25 mL DW before SPE analysis. The SPE procedure was repeated with samples spiked with iAs(V) at the 1 µg/L and 10 µg/L concentration levels.

3. Results and discussion

3.1. Efficiency of MW-assisted acid digestion and EDXRF analysis of the digestion residues with special emphasis on dissolution of As

Generally, the investigated *Ayurvedic* formulations could be readily dissolved by MW-assisted acid digestion (Table 1). As expected, the amount of the residues were greater in the case of the mineral formulations. Since large As content in form of realgar was expected in

the *Pama Malam* ointment according to the information leaflet accompanying this product, MW-assisted acid digestion was avoided in order to prevent persistent contamination of the quartz microvials and PTFE vessels. Therefore, the *Ayurvedic* ointment was analyzed by EDXRF. Moreover, the obtained residues after MW-assisted acid digestion were also analyzed by EDXRF to check the digestion efficiency. The acquired X-ray spectra can be seen in Figure S2 (Appendix). The EDXRF spectra of the ointment confirmed that As was the main component in this sample (Figure S2 in Appendix). In the case of the rest of the residues, As could not be detected. Taking into consideration the LOQ values of this benchtop equipment, this means that the As concentration in the residues were below some mg/kg. Therefore, the applied MW-assisted method can be considered successful for the majority of the investigated CAMs.

3.2. Determination of toxic element concentration in some Ayurvedic formulations

In the six different *Ayurvedic* products purchased from India *via* Internet and one bought in Hungary, determination of the concentration of Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Sn, Ba, Hg and Pb was achieved by MW-assisted acid digestion and ICP-SF-MS. Nine out of these 12 elements, i.e., Cr, Mn, Fe, Cu, Zn, Ba, Hg and Pb could be determined in all *Ayurvedic* formulation. Concentration of the elements covered a wide range from μg/kg to mg/kg (Table 2 and Figure S3 in Appendix). For Pb, the counts acquired for each three isotopes were summed, then related to the sum of the IS counts of In and the concentration was calculated by using a calibration curve plotted applying the same principle of count summing. The LOQ was calculated as 10 times the standard deviation of the signal of each element in the MW-assisted blank samples divided by the corresponding slope (Table 2).

The elemental composition of the two bovine colostrums was similar. Cadmium could not be detected in these samples. For both samples, Zn was present in the greatest

concentration: 50.4 ± 0.3 mg/kg in *Colostrovita vs.* 53.5 ± 0.5 mg/kg in *Kudos Colostrum.* Among the other formulations, the Asiatic pennywort and the herbomineral product (*Swasari Ras*) had remarkable Zn concentrations. Presence of As in traces (0.081 ± 0.029 mg/kg) in the *Kudos Colostrum* sample constituted the most important difference between the two similar samples as in the other colostrum As could not be detected (Figure 2a). Except for Pb (5.35 ± 0.08 mg/kg in *Kudos Colostrum vs.* 1.26 ± 0.02 mg/kg in *Colostrovita*), the elemental concentration data obtained for colostrums were relieving since these products are used for strengthening the immune system.

All investigated elements could be determined in the Asiatic pennywort and herbomineral formulations. Manganese (364 ± 15 mg/kg), Fe (4.96 ± 0.18 g/kg), Cd (0.71 ± 0.02 mg/kg) and Ba (83.2 ± 1.1 mg/kg) concentration in the Asiatic pennywort were the greatest for these elements among the investigated samples. However, the As (0.41 ± 0.05 mg/kg) and Hg (0.010 ± 0.001 mg/kg) concentrations in these samples were relatively low (Figure 2a). Moreover, this capsule formulation proved to be inhomogeneous. Thus, the As concentration in another composite sample was about the double (0.99 µg/kg *vs.* 0.41 µg/kg).

The elemental composition of the herbomineral compound originating from two different lots was quite similar except for Cu, Cd, Sn, Hg and Pb. The greater differences observed for the aforementioned elements were mainly due to the lower concentration values. The differences between the element concentration for Cu, Cd, Sn, Hg and Pb was 17%, 19%, 59%, 34% and 24 %, respectively. For the rest of the elements, this variation was less than 10%.

The As and Hg concentration in the white arsenic and red cinnabar containing *sindoor* formulation was the greatest and the second greatest among the investigated products with a concentration of 106 ± 13 g/kg and 1.97 ± 0.01 g/kg, respectively. The concentration of the

other investigated elements was quite low which may be also due to the phase change (e.g., sublimation) applied during its preparation.

The ICP-MS measurements also proved that the main element of the *kushta* formulation is Sn (197 ± 2 mg/kg). Except for Cu (39.8 ± 0.8 mg/kg) and Pb (60.9 ± 0.7 mg/kg), the concentration of the other investigated elements was low. Arsenic could not be even quantified in this sample.

It is difficult to compare our results with literature data due to the great diversity of *Ayurvedic* products. However, Khandpur *et al.* [22] investigated eight different *Ayurvedic* formulation with an As concentrations ranging between 5 – 248 mg/kg prescribed daily once to an eleven years old girl. The As concentration in the blood sample taken from the patient was 0.202 mg/L, while the normal values are usually <0.06 µg/L. The symptoms improved after the discontinuation of the treatment.

Saper *et al.* investigated 70 and 193 different *Ayurvedic* formulations sold in Boston (USA) [23] or via Internet [24] by X-ray fluorescence. Fourteen out of the investigated 70 samples contained Pb, Hg and As in quantifiable amounts. Moreover, the Pb concentration ranged between 5 mg/kg and 37 g/kg in 13 samples, that of Hg between 28 mg/kg and 10.4 g/kg and As between 37 mg/kg and 8.13 g/kg in each sample [23].

Giacomino *et al.* [25] determined the essential and toxic element concentration in five different *Ayurvedic* formulations with large Hg concentration determined by ICP optical emission spectrometry and graphite furnace atomic absorption spectrometry (for Cd and Pb) after MW-assisted acid digestion [25]. The As and Hg concentration in the samples ranged between 0.04 and 771 mg/kg and 13.27 – 28.26 mg/kg, respectively. Such products had also been analyzed by voltammetry [26], instrumental neutron activation analysis [27], XRF spectroscopy [23, 24, 28] or inductively coupled plasma mass spectrometry (ICP-MS) [18]. The XRF has the advantage of being non-destructive, but it cannot be applied to elements

present at trace levels, while ICP-MS has an extremely high sensitivity, but it requires an expensive instrumentation and considerably more laborious sample preparation.

3.3 Investigation of As bioaccessibility

The bioaccessibility of As from the purchased samples was also investigated in HCl containing pepsin as well as pancreatin in phosphate buffer of pH = 6.8 simulating gastric and duodenal juices, respectively. These model fluids and their pH did not alter considerably the extraction efficiency (Figure 2b). Mass balance was not established in order to protect the analytical equipments from cross-contamination arising from the large concentrations of potentially toxic elements characteristic to these formulations. However, the total As content of the Asiatic pennywort formulation was almost completely available for both extraction media (Figure 2b). The As extraction rate in the two identical products originating from different lots was also remarkably large and the results were in good agreement ($\pm 5 - 20\%$) (Figure 2b). Arsenic could be extracted in about 10-20% from the mineral formulations containing As(III) oxide and red HgS as well as α-As$_4$S$_4$ and HgS (Figure 2b). This can be explained by the fact that As(III) oxide and sulfides are not readily soluble in HCl solutions because of being (thio)acid anhydrides. Therefore, their dissolution can be expected only in alkaline media. Due to the large total As concentration, however, As intake from these two latter products may be high. For the As(III) oxide and red HgS containing as well as for the α-As$_4$S$_4$ and HgS samples, the daily As intake exceeded the 3.0 µg/BW kg/d intake recommended by WHO [29] by a factor range comprised of $11.1 - 21.4$ and $6.5 - 26.2$, respectively (Table 3). Although these toxic products could be purchased without prescription, their application should be restricted to such drastic pathological conditions like syphilis, malaria, cholera, asthma, pneumonia, anorexia etc., for short time periods (1-2 months) and under a strict medical supervision (Table 2 and Table S1 in Appendix).

Up to our knowledge, few complex analytical data on the possibly toxic element concentration in *Ayurvedic* formulations have been reported [30-32]. By investigating the bioaccessible content of toxic elements in 17 different *Ayurvedic* formulations purchased *via* Internet, Italian pharmacies and Indian local markets by incubation of the samples in suspensions simulating gastric and duodenal juices, a remarkable dissolution was observed for the investigated toxic elements. The concentration of As potentially adsorbed during the digestion of two products was greater than the maximum admissible daily intake for this element [21, 25].

Koch *et al.* [30] mapped the elemental content of CAMs by X-ray absorption near edge structure (XANES) focusing also on the bioaccessible fractions of As and Pb by incubation in model suspensions containing either pancreatin or bile extract. For As determination, the ICP-MS technique proved to be suitable. Interestingly, *bhasma* formulations that are supposed to decrease As toxicity due to strong covalent bound between As(III) and S, facilitated dissolution of As in the bioaccessible fractions. The reason behind this phenomenon was the oxidation of As(III) and subsequently, replacement of sulfur atoms by oxygen ones. This outcome was supported by the XANES measurements. Moreover, the dietary As intake estimated by using the bioaccessible As concentration values was greater in several cases than the daily tolerable limit set by the California Proposition 65 [33]. The extraction efficiency by modeling bioaccessibility showed big differences. This pointed out the importance of the proper choice of the extraction method, however there are no *in vivo* data available to support any of the procedures applied for assessment of toxic element bioaccessibility [31].

Bolan *et al.* [32] reported on an exhaustive sequential extraction procedure according to Tessier *et al.* applied usually for soils [34] as well as bioaccessibility study by using simulated gastric and duodenal juices on the As content of rice and several CAMs, i.e., herbal and *Ayurvedic* formulations. The available As concentration strongly depended on the procedure

applied. The authors also pointed out at the lack of an adequate procedure for bioaccessibility studies. However, pancreatin is one of the most popular compounds for the simulation of duodenal juice [30, 35-38]. Thus, about 19% of total As represented the readily soluble fraction that together with the carbonatic one correlated with the bioaccessible percent. However, no thread was detected by estimating the daily dietary intake.

3.4. Arsenic discrimination in phosphate-rich bioaccessible fractions of some Ayurvedic formulations

Suitability of IE-SPE for As speciation was tested in two *Ayurvedic* medicines of moderate and two other ones with large As contents for *in vitro* bioaccessibility simulating the conditions present in the human intestinal tract by using sample amounts corresponding to their maximum daily intake. The *in vitro* bioaccessibility test performed with model gastric juice failed due to the low pH of this type of medium that protonates iAs(V) possibly present in the samples even when attempting to raise the pH of the incubated suspensions with ammonia. However, oxidation of the initial iAs(III) in the samples to iAs(V) can occur at slightly alkaline pH in the presence of an oxidizing agent. Therefore, we focused on the As speciation in the duodenal juice simulating system.

Because of the high organic material content of the resulting suspensions which would be coagulated during the elution of the iAs(V) fraction, MW-assisted acid digestion was applied prior to the ICP-MS measurements. So far, it has been reported that proteins can be retained by SPE cartridges when sample clean-up is needed. Furthermore, it was reported that pancreatin decreases the quantifiable amounts of iAs(III) at HPLC separation [39]. Due to this digestion step and proper dilution rate applied for the subsequent ICP-MS measurements, As could not be detected in the *Ayuvedic* formulations subjected to incubation in duodenal juice simulating fluid and SPE for the two formulations containing As in moderate concentration

when the investigated amount corresponded to the maximum daily intake amount. Nevertheless, when these two investigated *Ayurvedic* formulations were spiked with 150 µg/kg iAs(V) and incubated with the duodenal juice simulating suspension, the recovery rate of As was between 70-84% after elution from the IE-SPE cartridges (Figure 3a). Although the recovery rate of As was not remarkably high, a proper mass balance could be set up. Thus, about 25% - 40% of the spiked amount could not be retained by the microcartridges. This breakthrough might be due to either competition of phosphate ions with iAs(V) or blocking of the active sites of the ion exchange (IE) material by the high protein content of the model suspension. Another factor affecting the efficiency of separation may be the pH of the incubation medium. Higher pH values favor the acid dissociation of iAs(III). We have previously demonstrated that this alternative speciation method deteriorates the selectivity starting from pH = 9 [17]. However, the pH of the suspension used in this study was set to 6.8, where the acid dissociation of iAs(III) is negligible. Reduction of iAs(V) to iAs(III) under the experimental conditions is unlikely. The standard addition method for As determination was not considered because it would have increased the sample number and, consequently, the operational costs of the analysis.

In order to check the influence of phosphate ions on the retention of iAs(V), the experiment was repeated without its addition to the model suspension. Thus, the absence of the phosphate salt of the incubation medium increased the recovery of iAs(V) to about 80% (Figure 3a) and the iAs(V) breakthrough rate decreased, too. When 150 µg/L aqueous iAs(V) standards were also subjected to the entire procedure, a similar trend for As speciation could be observed (Figure 3a). Thus, the sample matrix and pancreatin proved to not alter considerably the efficiency of the separation step. The presence of NaOH did not influence the iAs(V) recovery. This is in agreement with the previous observations where competition between hydroxide and iAs(V) ions for the active sites of the IE material occurred at much

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higher alkaline pH values [17]. By decreasing the spike concentration level of iAs(V) to 75 μg/L, mass balance could not be established because As could not be quantified in the fraction collected after passing through the loaded sample. Moreover, the As and iAs(V) recoveries in the presence of phosphate ions were 74% and 67%, respectively, on average, while the absence of these ions in the incubation medium increased retention of iAs(V), the recovery being 82-87%, in this latter case (Figure 3b). It can therefore be concluded that the large phosphate content of the extraction medium limited the applicability of IE-SPE. However, As speciation in bioaccessible fractions is not a common procedure. Up to now, elemental speciation is usually performed directly on the solid samples by X-ray based techniques (mainly XANES, extended X-ray absorption fine structure, EXAFS) due to the large amounts of toxic elements contained in *Ayurvedic* formulations [30-32]. In another study, EXAFS was applied for CAMs in solid state of matter [32]. The predominant As species in herbal medicines were organic ones, while in the case of *Ayurvedic* formulations inorganic ones.

Finally, oxidation of iAs(III) could be observed by applying the optimum SPE conditions for the two *Ayurvedic* (herbo)mineral formulations with large As content. Thus, 6.3% and 33% of the bioaccessible As content was oxidized to iAs(V) in the white arsenic and red cinnabar as well as realgar and HgS containing mineral formulations, respectively.

3.5. Total As content and As speciation in a homeopathic product

For comparison purposes, a *homeopathic* medicine prepared by excessively diluting white As (10^{18} dilution rate performed in nine steps) and containing sucrose and lactose as matrix components was also included in the present study. Determination of total As in the product originating from two different lots as well as for the other monitored elements failed when MW-assisted digestion was applied. This is understandable due to the excessive dilution of white As used for preparation of the product, although the product that underwent the least

possible dilution rate had been purchased on purpose for our investigations. Therefore, the As concentration of the homeopathic product rather reflects the As contamination of the manipulation during its preparation. However, As determination was successful in the aqueous solution of the product because it was readily soluble in water. The investigation showed that the total As concentration of the different batches were in the 83.4 µg/kg – 147 µg/kg range. Thus, the mean total As concentration was about 106 ± 33 µg/kg (n = 5). The relatively greater SD value is a proof of the *inter*lot variation. The iAs(V) concentration was found to be varying between 16.3 and 28.1 µg/kg, while the iAs(III) one proved to be much more prone to fluctuations, ranging from 58.8 µg/kg up to 119 µg/kg. Since the total As determination was performed from an aqueous solution as well as As speciation was also a scope of the present study, the samples were spiked with 1 µg/L iAs(V) and subjected to IE-SPE. The recovery rate of total and iAs(V) was 97.0% ± 7.8% and 95.2% ± 3.1%, respectively.

By incubating the homeopathic product spiked with 10 µg/L iAs(V) in the model duodenal juice suspension, the recovery rate of total As and iAs(V) was about 70% and 50%, respectively (Figure 4). Moreover, about 20% of iAs(V) breakthrough was observed in the presence of phosphate ions. By repeating the experiment without addition of these competing ions, the recovery rates for total As and iAs(V) increased roughly by 10-12% for each fraction (Figure 4) and no breakthrough was observed. This outcome reinforces that the competing phosphate ions present in the incubation media in a concentration of 0.05 M are the limiting factor for the success of As speciation by IE-SPE in CAMs.

4. Conclusions

The potential health risks associated to the As intake from the bioaccessible fractions of the investigated *Ayurvedic* formulations have been pointed it in the present work. In a recent

review [40], i) identification of the introduction mechanisms of toxic elements into CAMs, ii) knowledge of the biogeochemical cycle of these elements especially in terms of metabolites; iii) development of suitable speciation and *in vitro* methods for studying bioaccessibility of toxic elements; iv) establishing correlation between elemental speciation and bioaccessibility as well as v) execution of clinical tests and implementation of stricter legislations were pointed out. So far, elemental speciation for *Ayurvedic* formulations characterized by elevated concentration of potentially toxic elements has only been performed in the solid state of matter by expensive X-ray techniques (commonly XANES, EXAFS). Although the success of the X-ray techniques is indisputable, more accurate information would be obtained by applying a suitable speciation procedure on the *in vitro* bioaccessible contents through incubation in model system mimicking the strong acidic gastric and the almost alkaline duodenal juices. Development of a cost-effective speciation method such as IE-SPE would be ideal for iAs speciation. However, the low bioaccessibility of As, the extreme pH conditions and complexity of the sample/extractant matrix with special emphasis on the phosphate ions competing with iAs(V) for the IE sites represent a challenge that could be only partially solved in the present manuscript. Moreover, direct HPLC analysis of As speciation on pancreatin containing suspensions is also hard to be accomplished without filtration through membrane filters. Nevertheless, this novel IE-SPE approach is more informative than extrapolation of speciation results by expensive X-ray based techniques performed on solid state of CAMs, e.g., allowing estimation of the oxidation rate of iAs(III) to the less toxic iAs(V) species in the bioaccessible content. In addition,

'Appendix A. Supplementary data' and 'Supplementary data to this article can be found online at doi:...'.

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Figure captions

Figure 1. Flow chart of the analytical procedures applied for the complementary and alternative medicines included in the present study. Error bars represent the standard deviation of the results (n = 3).

Observations: 1 = sample mass of 200 – 500 mg (n = 3) chosen according to the estimated daily intake; 2 = sample mass (n = 5) for the homeopathic formulation was 500 mg; 3 = EDXRF analysis of the *Ayurvedic* ointment directly; 4 = MW-assisted digestion for IE-SPE analysis;

Abbreviations: ICP-SF-MS = inductively coupled plasma sector field mass spectrometry; IE = ion-exchange; MW = microwave; R = resolution; SPE = solid phase extraction.

Figure 2. Total concentration (a) and bioaccessible fraction of As (b) in *Ayurvedic* formulations (for the latter incubation with gastric and duodenal juices simulating suspensions). Error bars represent the standard deviation of the results (n = 3).

Color codes: black = mineral; brown = animal; dark yellow = herbomineral; green = herbal formulation.

Figure 3. Recovery rates of total As and iAs(V) by IE-SPE-ICP-SF-MS from either 150 µg/L iAs(V) aqueous standard solution or two *Ayurvedic* formulations spiked with 150 µg/L iAs(V) (a) as well as 75 µg/L iAs(V) standard solutions (b) subsequently incubated in duodenal juice simulating medium with or without hydrogen phosphate ion and sodium hydroxide addition. Error bars represent the standard deviation of the results (n = 3).

Plus and minus signs symbolize the presence and absence of one of the components of the simulated duodenal juice, respectively.

Figure 4. Recovery rates of total As and iAs(V) by IE-SPE-ICP-SF-MS from a homeopathic medicine spiked with 10 µg/L iAs(V) standard solution subsequently incubated in duodenal juice simulating medium with or without hydrogen phosphate ion and sodium hydroxide addition. Error bars represent the standard deviation of the results (n = 3).

Plus and minus signs symbolize the presence and absence of one of the components of the simulated duodenal juice, respectively.
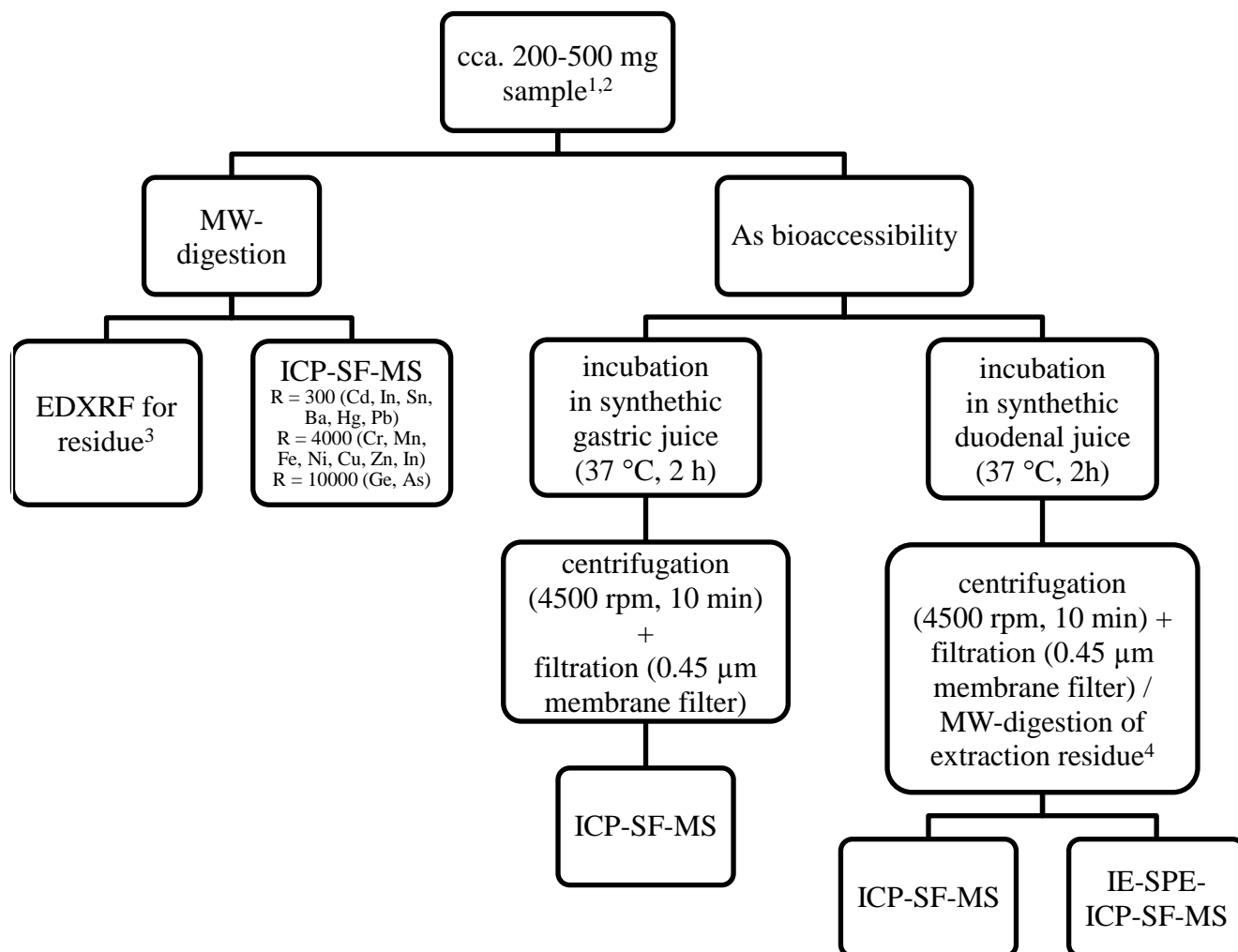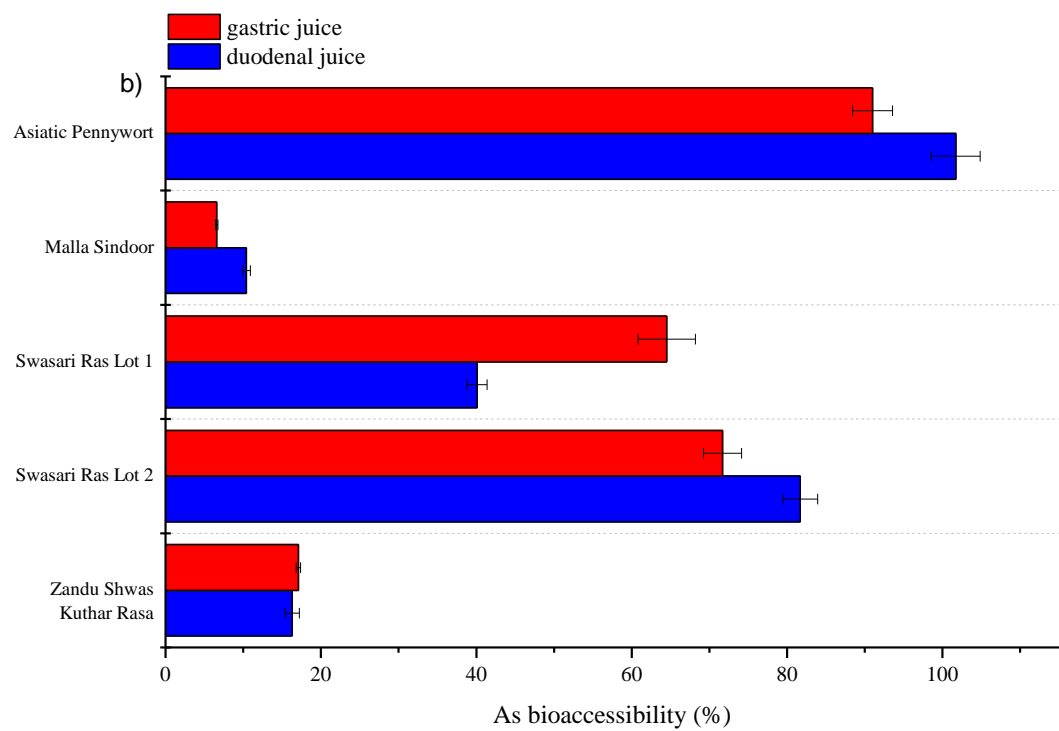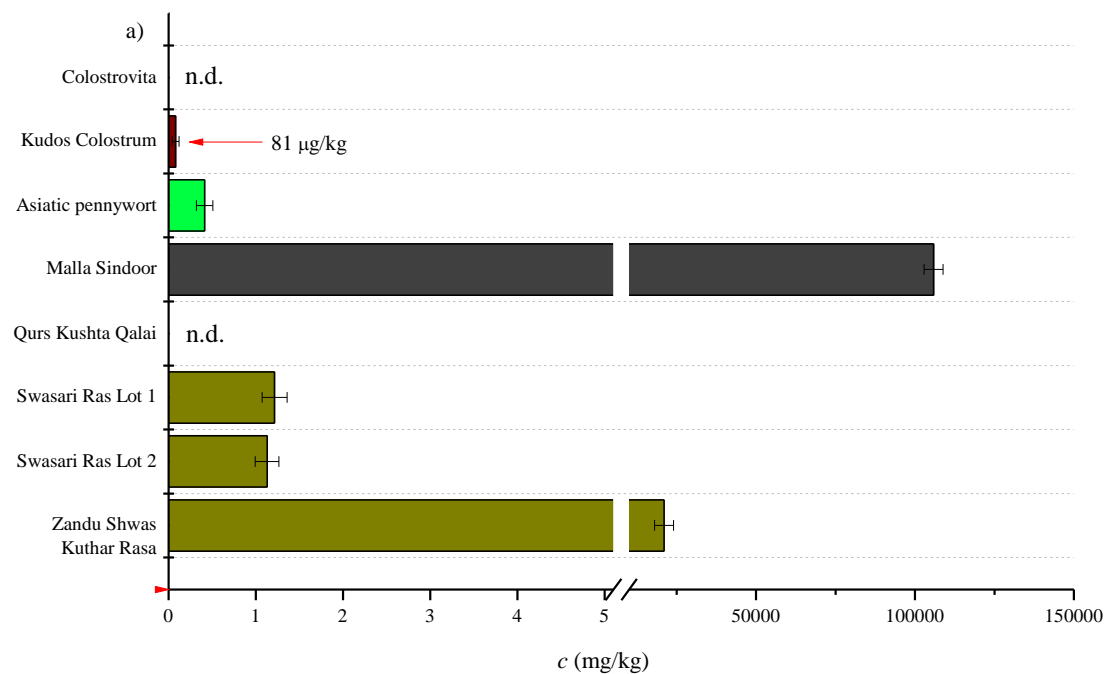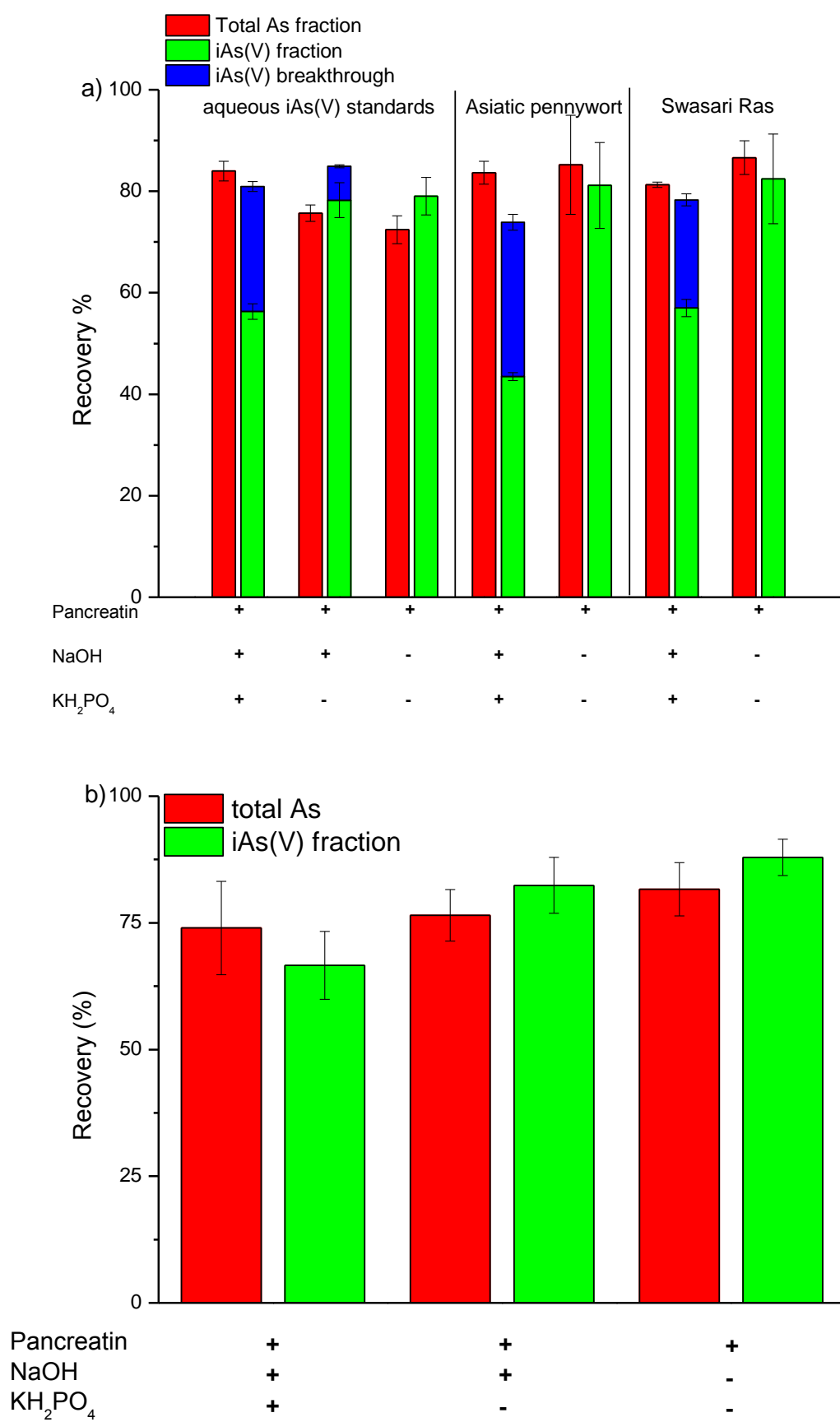
Figures

Figure 1.

cca. 200-500 mg sample[1,2]

MW-digestion

As bioaccessibility

EDXRF for residue[3]

ICP-SF-MS
R = 300 (Cd, In, Sn, Ba, Hg, Pb)
R = 4000 (Cr, Mn, Fe, Ni, Cu, Zn, In)
R = 10000 (Ge, As)

incubation in synthethic gastric juice (37 °C, 2 h)

incubation in synthethic duodenal juice (37 °C, 2h)

centrifugation (4500 rpm, 10 min) + filtration (0.45 µm membrane filter)

centrifugation (4500 rpm, 10 min) + filtration (0.45 µm membrane filter) / MW-digestion of extraction residue[4]

ICP-SF-MS

ICP-SF-MS

IE-SPE-ICP-SF-MS

Figure 2

Figure 3

Figure 4
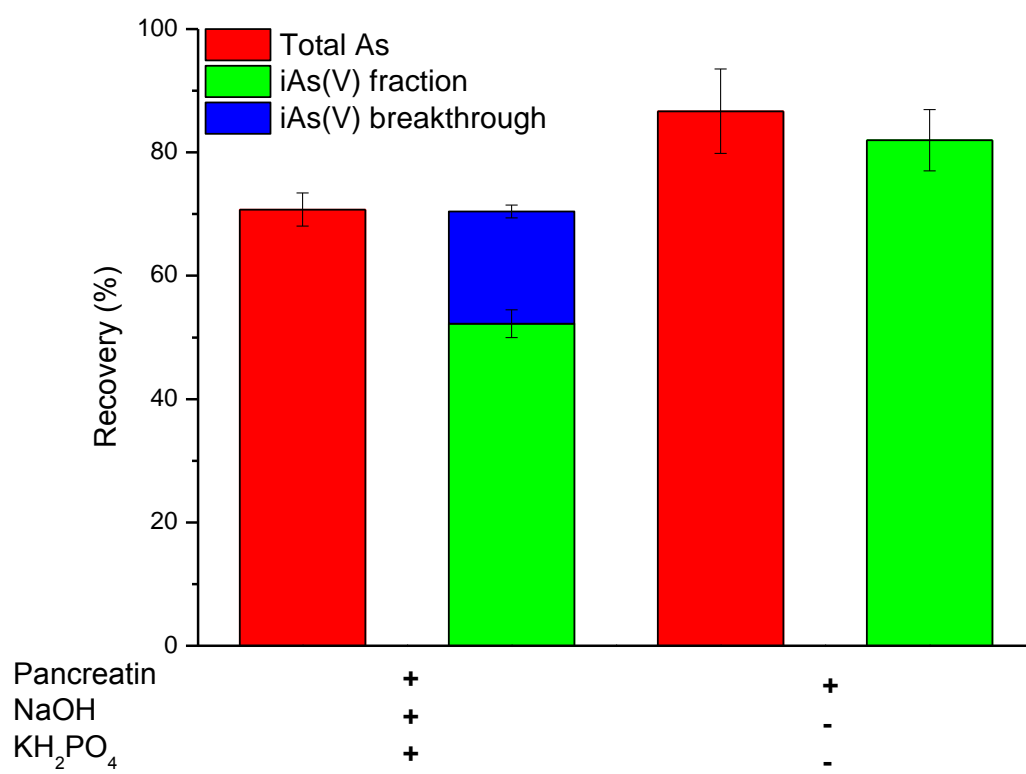
Tables

Table 1. Basic information about the complementary and alternative medicines investigated and efficiency of MW-assisted acid digestion expressed as digestion residue percentage

Sample brand name	Sample classification	Main ingredient	Therapeutic indication	Residue (% by weight)
Acidum arsenicosum anhidricum 9 CH	n.a.	lactose, sucrose	gastric and respiratory system disorders	-
Colostrovita	animal	n.a.	immunity strengthening	-
Kudos Colostrum		n.a.		-
Gotu Kola (Asiatic pennywort)	herbal	n.a.	improving memory, decreasing fatigue and depressive mood. Sleep disorders.	5.6
Qurs Kushta Qalai	mineral	n.a.	malaria, cholera, syphilis, pneumonia, paralysis; asthma;	58
Pama Malam	herbomineral	orpiment, realgar	scabies, eczema, ring-worm, psoriasis, barber's itch	n.a.
Malla Sindoor	mineral	white As, red cinnabar	disorders of male reproductive system	42
Swasari Ras (lot 1 & 2)	herbomineral	biotite, gypsum	asthma, cough, sinusitis, hay fever, headache	3.4
Zandu Shwas Kuthar Rasa	herbomineral	realgar, borax	asthma	-

n.a. = not applicable / not available

Table 2. Concentration range of potentially toxic elements in the investigated *Ayurvedic* formulations and limit of quantitation (LOQ) calculated as 10σ by ICP-SF-MS.

Monitored element	Concentration range		LOQ (mg/kg)
	minimum (mg/kg)	maximum (mg/kg)	
Cr	0.122	267	0.002
Mn	0.086	363	0.004
Ni	0.034	118	0.004
Cu	0.800	39.7	0.002
Zn	3.56	224	0.012
Cd	0.006	0.71	0.004
Sn	0.071	197	0.007
Ba	0.154	83.2	0.001
Pb	1.20	60.9	0.002
	minimum (mg/kg)	maximum (g/kg)	
Fe	5.84	4.96	0.053
As	0.081	106	0.003
Hg	0.004	6.33	0.0004

Table 3. Estimated daily As intake from the investigated complementary and alternative medicines taking consideration the daily doses and body weight (BW) of 70 kg.

Sample	Extraction media	daily dose (g)		estimated daily As intake µg/BW	
		minimum	maximum	minimum	maximum
Acidum arsenicosum anhydricum 9 CH[a]	gastric duodenal	0.25	0.50	0.0004	0.0008
			Sum:	**0.0008**	**0.0016**
Asiatic pennywort	gastric	1.00	2.00	0.0135	0.027
	duodenal			0.0150	0.030
			Sum:	**0.0285**	**0.057**
Malla Sindoor	gastric	0.13	0.25	20.4	39.3
	duodenal			13.0	25.0
			Sum:	**33.4**	**64.3**
Swasari Ras lot 1	gastric	1.00	3.00	0.0113	0.0338
	duodenal			0.0070	0.0209
			Sum:	**0.0183**	**0.0547**
Swasari Ras lot 2	gastric	1.00	3.00	0.0131	0.0394
	duodenal			0.0116	0.0347
			Sum:	**0.0247**	**0.0641**
Zandu Shwas Kuthar Rasa	gastric	0.20	0.78	9.6	38.3
	duodenal			10.0	40.2
			Sum:	**19.6**	**78.5**

[a] estimation based on the total As concentration in the aqueous solution

SUPPLEMENTARY MATERIAL

Feasibility of arsenic speciation by ion-exchange solid phase extraction inductively coupled plasma mass spectrometry for *in vitro* bioaccessible contents of *Ayurvedic* formulations [‡]

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Figure S1. Brand names and origin of *Ayurvedic* formulations included in the present study
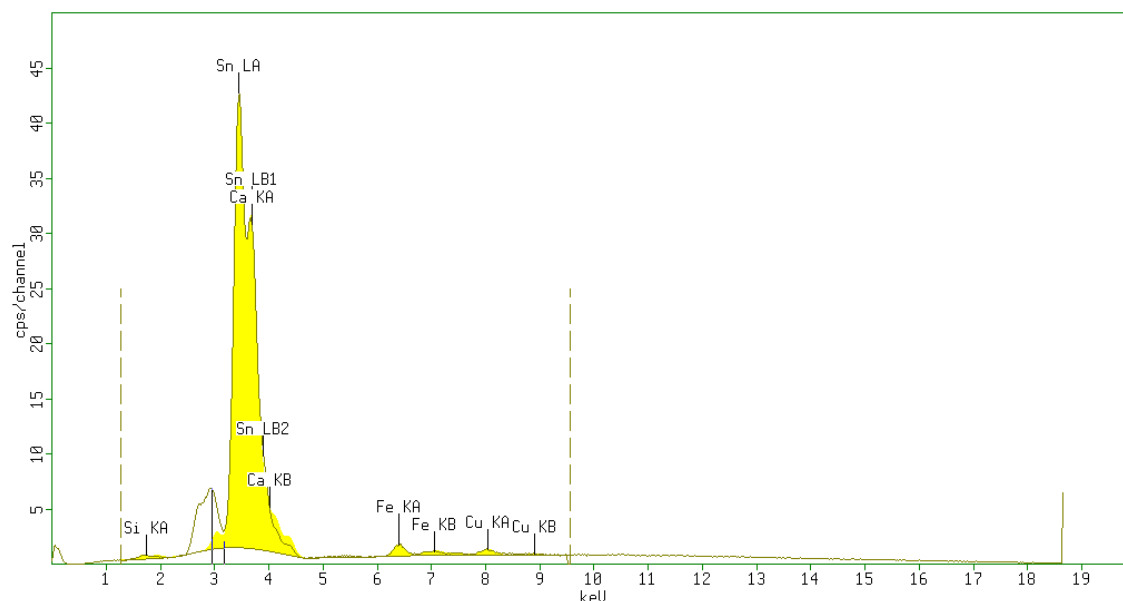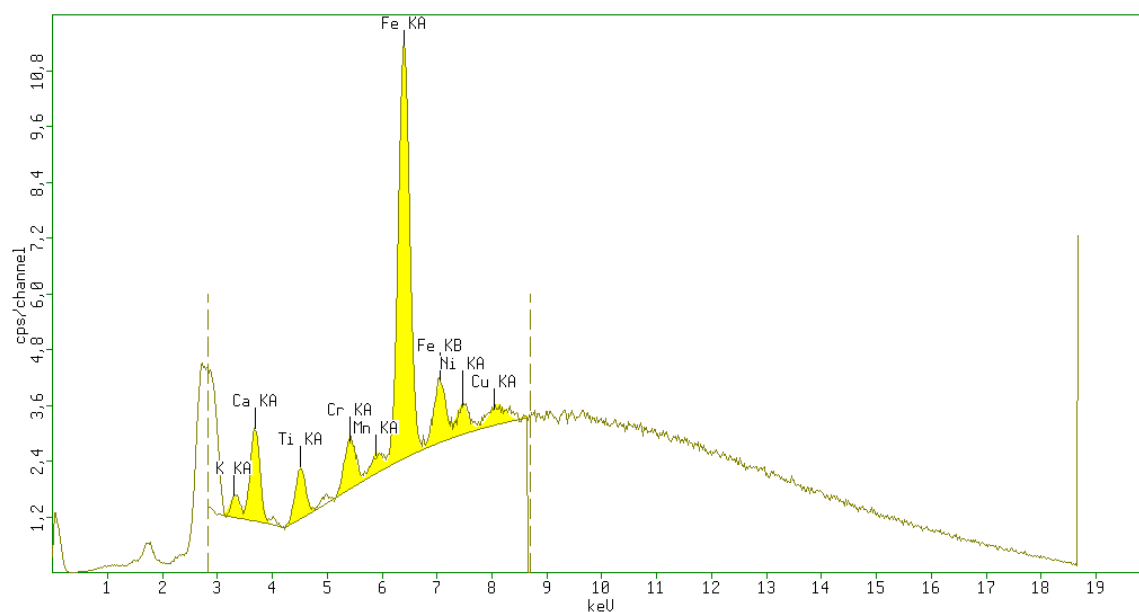
Figure S2

a)
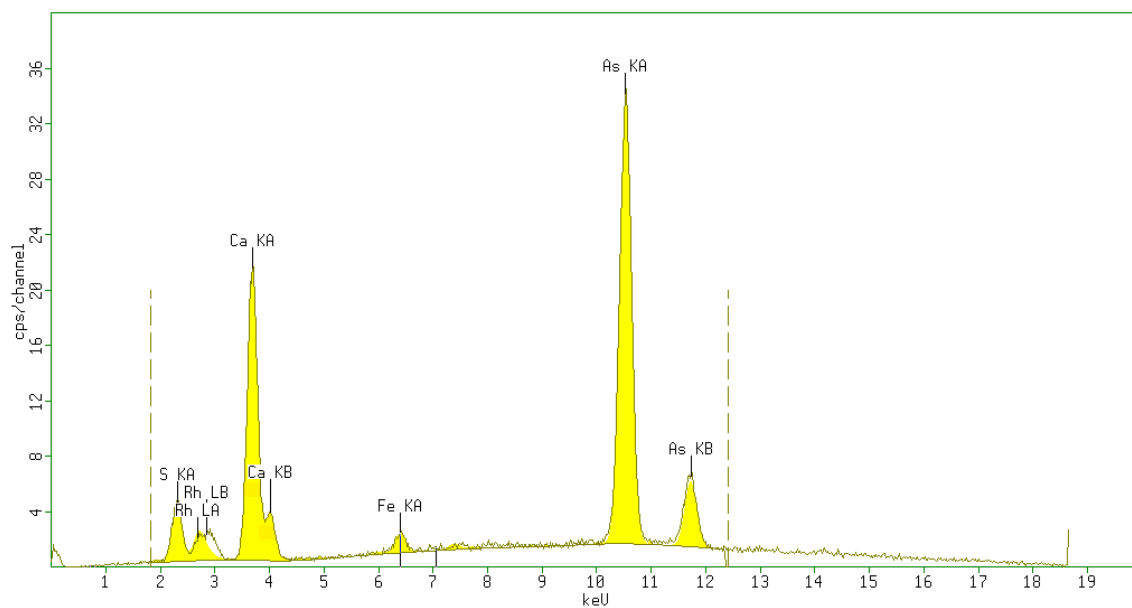
b)
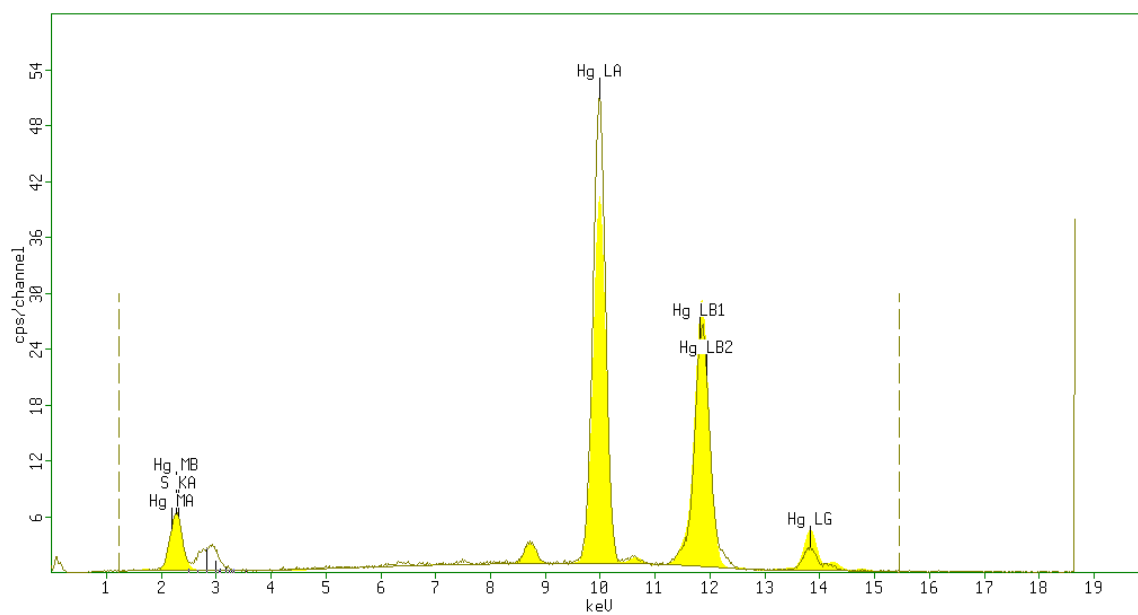
c)

d)

Figure S2. X-ray fluorescent spectra of realgar containing *Ayurvedic* ointment (a) and 5-50% by weight residues resulted after MW-assisted acid digestion: white arsenic and red cinnabar containing *sindoor* formulation (b); *Qurs Kushta Qalai* (c); Asiatic pennywort (*Centella asiatica*) (d).
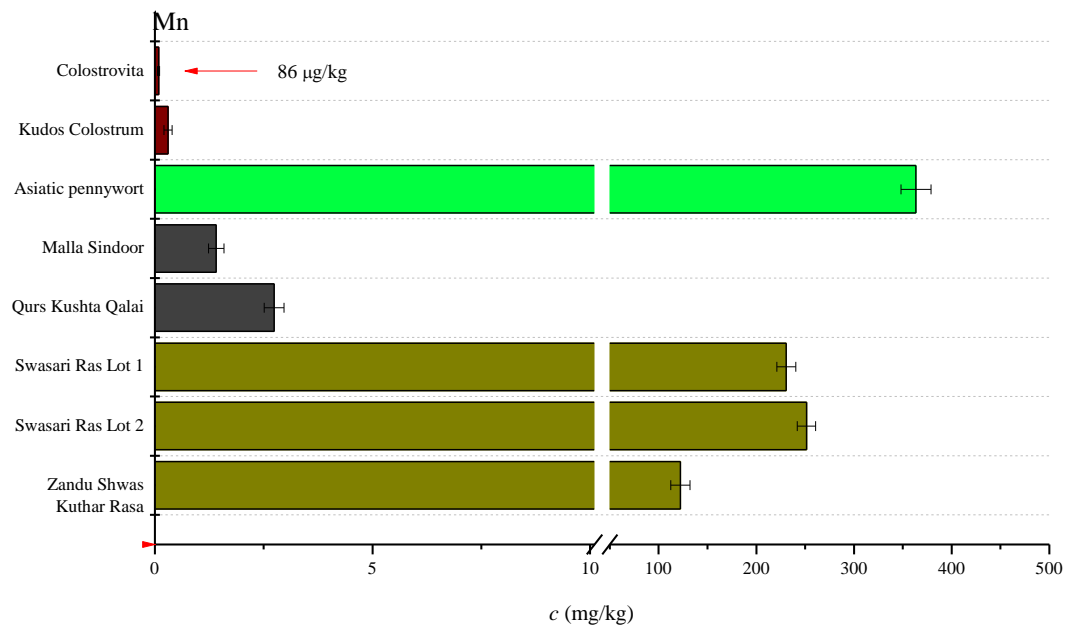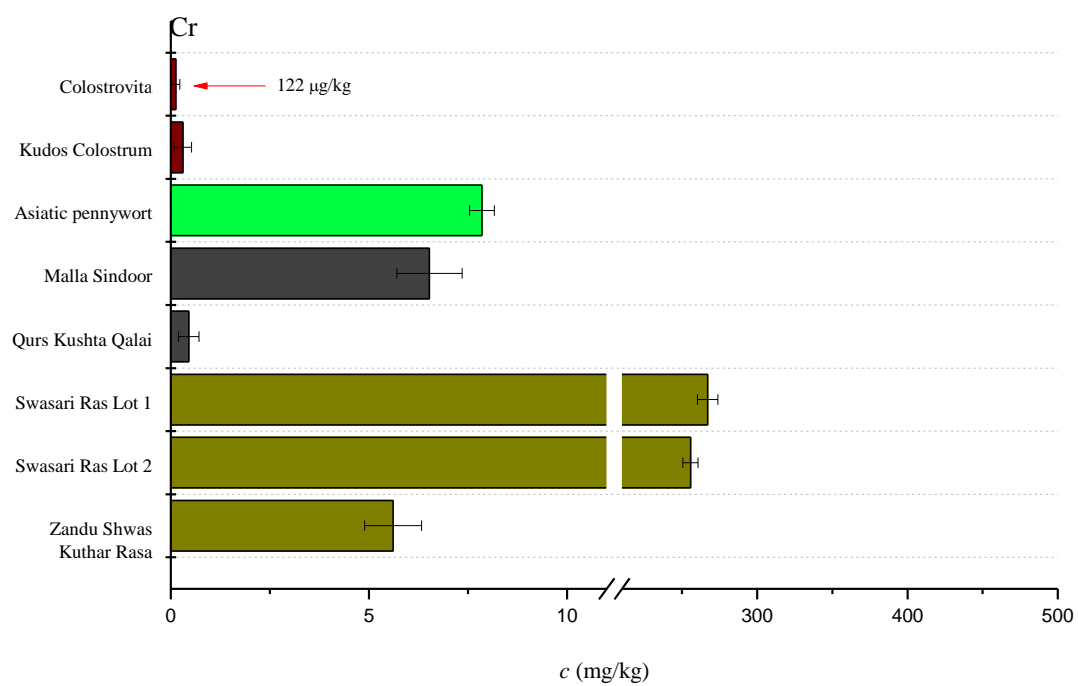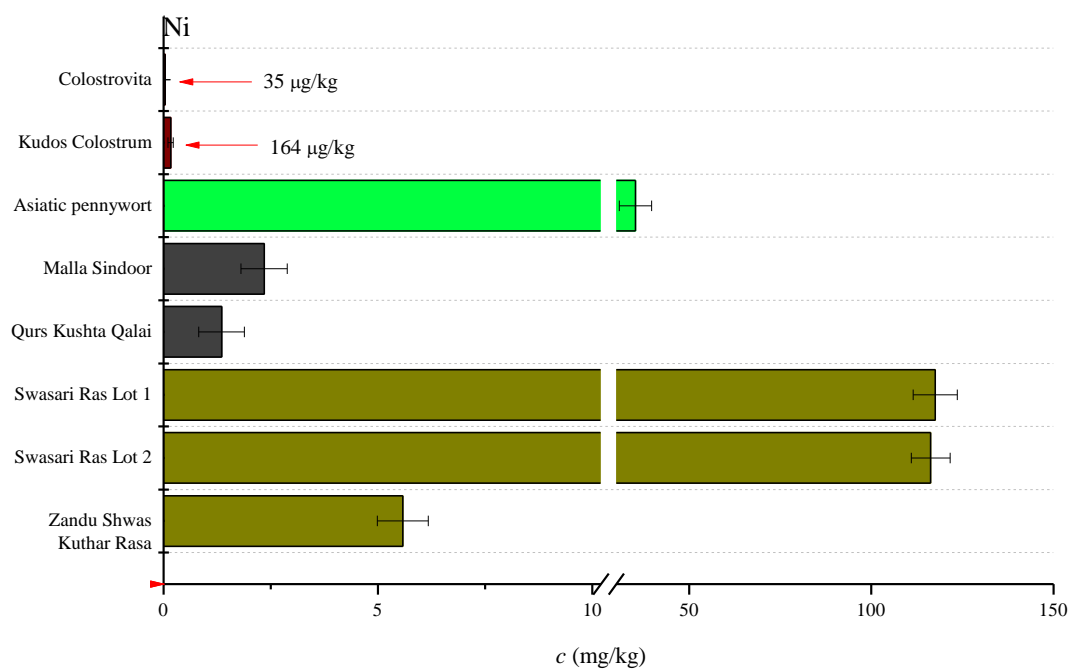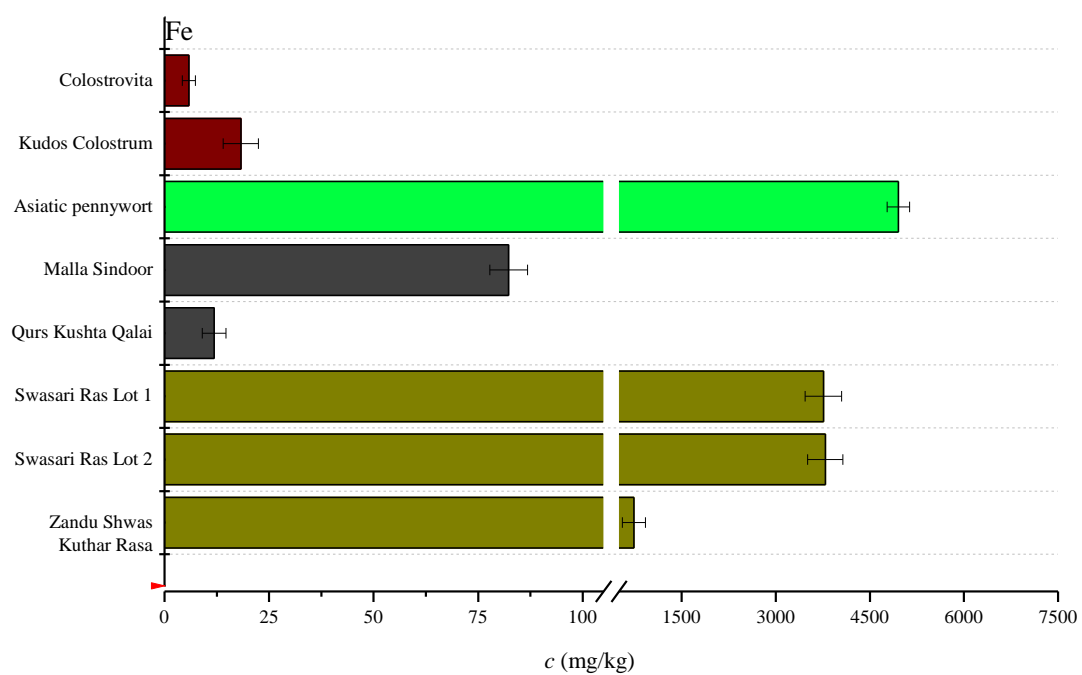
Figure S3

Cd

Colostrovita	n.d.
Kudos Colostrum	n.d.
Asiatic pennywort	
Malla Sindoor	6 µg/kg
Qurs Kushta Qalai	2 µg/kg
Swasari Ras Lot 1	
Swasari Ras Lot 2	
Zandu Shwas Kuthar Rasa	12 µg/kg

c (mg/kg)

Sn

Colostrovita	152 µg/kg
Kudos Colostrum	
Asiatic pennywort	71 µg/kg
Malla Sindoor	
Qurs Kushta Qalai	
Swasari Ras Lot 1	
Swasari Ras Lot 2	236 µg/kg
Zandu Shwas Kuthar Rasa	

c (mg/kg)
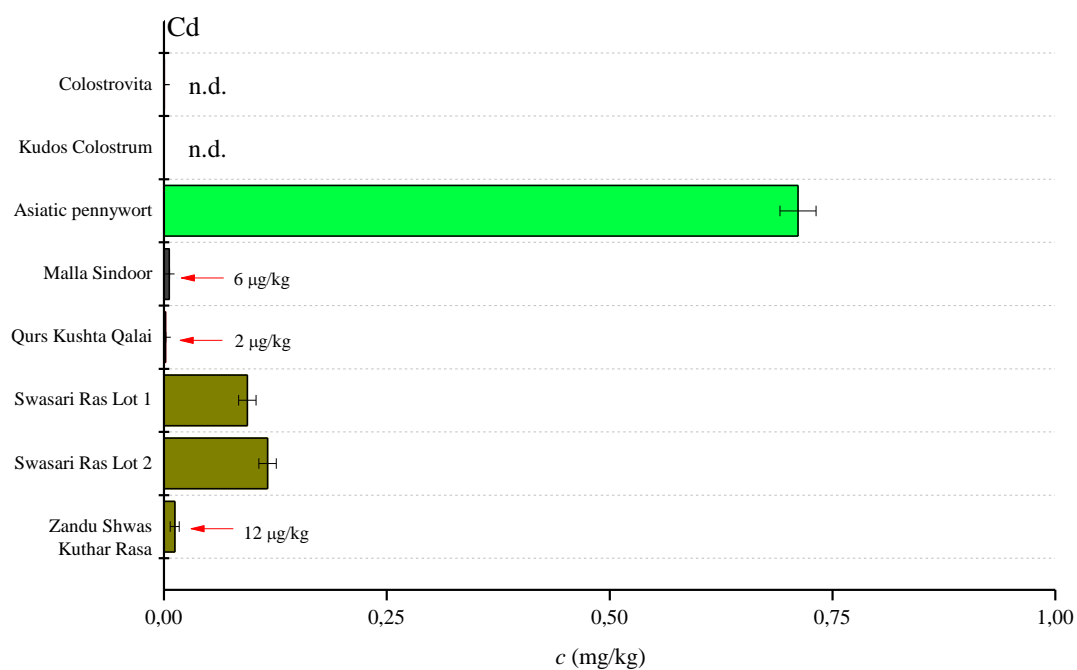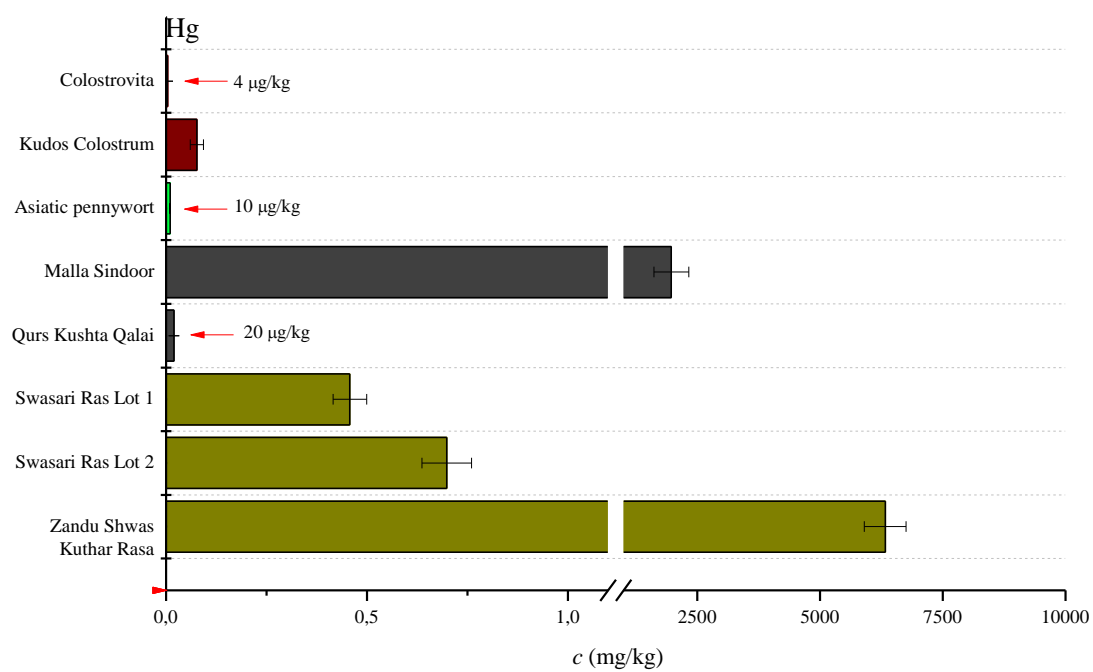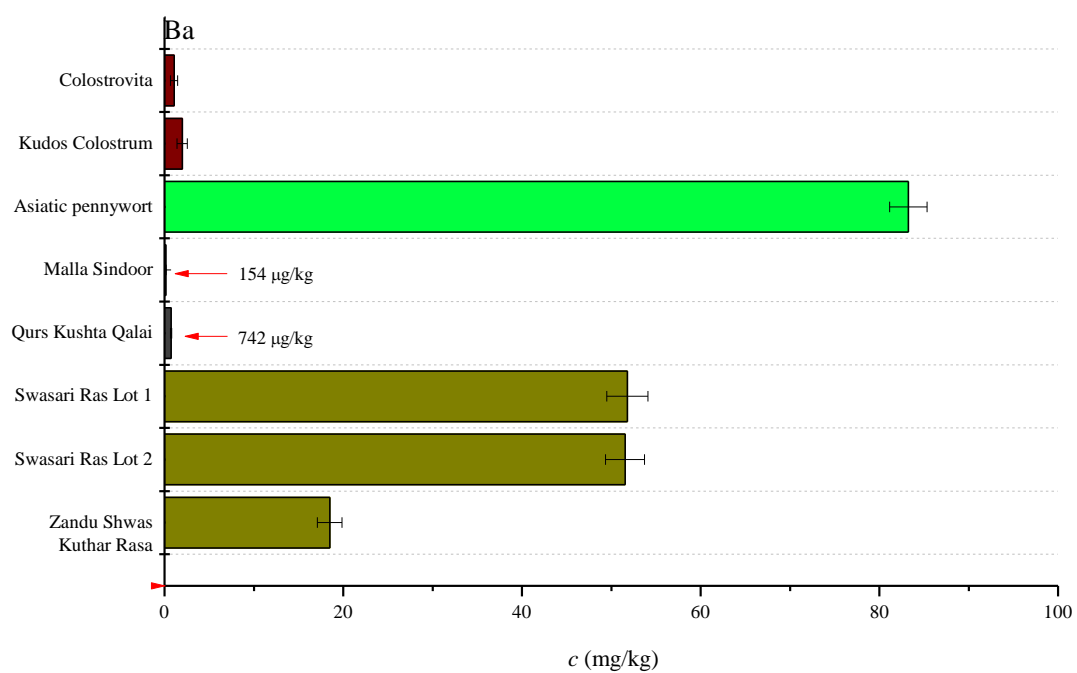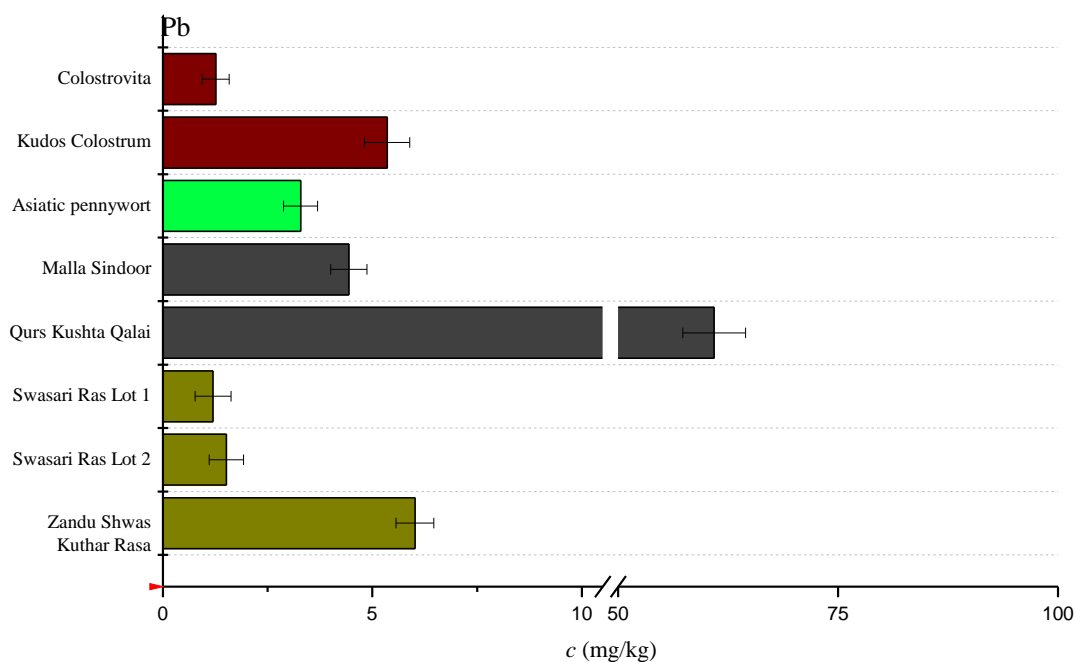
43
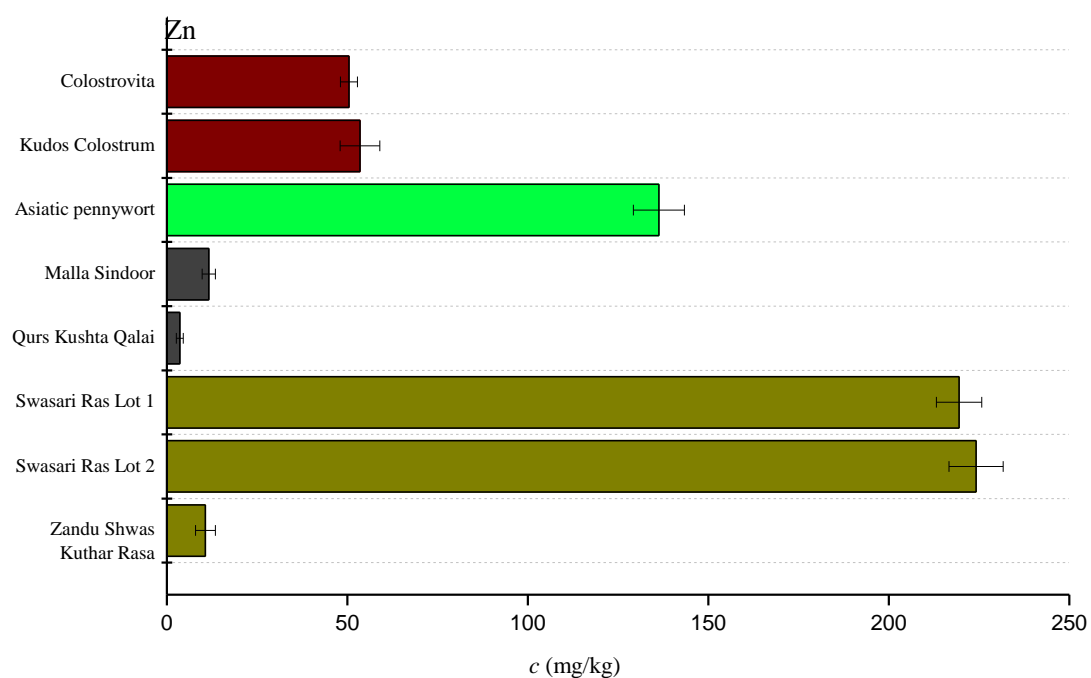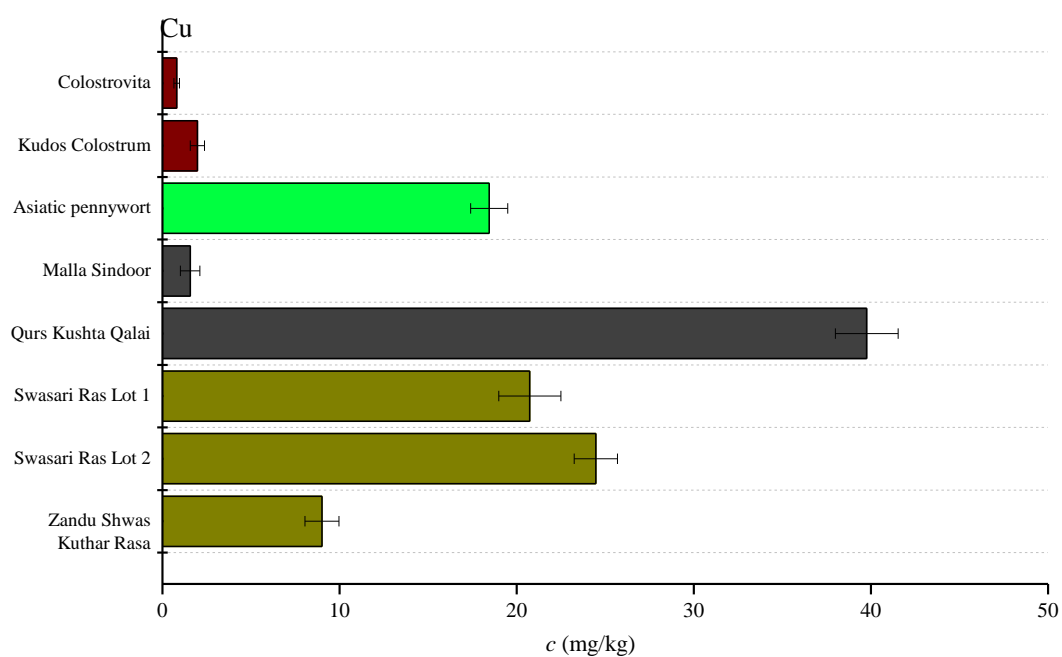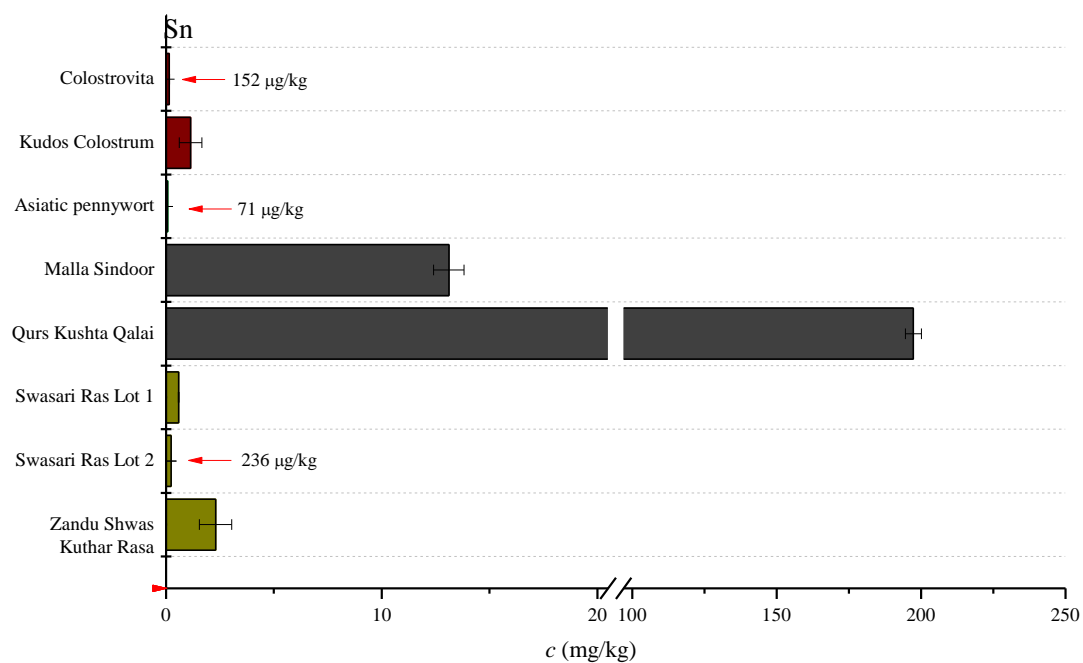
Figure S3. Total concentration of potentially toxic elements (represented in increasing atomic number) in *Ayurvedic* formulations subjected to MW-assisted acid digestion and ICP-SF-MS measurements. Error bars represent the standard deviation of the results (n = 3).

Color codes: black = mineral; brown = animal; dark yellow = herbomineral; green = herbal formulation.

Table S1. Detailed characteristics of the investigated complementary and alternative medicines

Product name	Presentation (size)	Ingredients declared by producer	Producer/Country/State /District, Department/Settlement	Acquisition source	Therapeutic indication	Daily dose	Manufacturing /Expiry date	Nominal mass (g)
Acidum arsenicosum anhydricum 9 CH[*]	pills (n=80)	0.04 g lactose/ 5 pills	Laboratoires Boiron, (France/-/Auvergne-Rhône-Alpes/Messimy)	store (Hungary)	gastric system and respiratory system disorders	1-2× 5 g pills daily	n.a./ 12/2020	4
Colostrovita	capsules (n=60)	100% bovine colostrums powder and gelatin (capsule shell)	Cure Nutraceutical Pvt. Ltd., India, Gujarat, Patan, Siddhpur	Internet	immunity strengthening	2× 2 capsules daily for adults; 2× 1 capsule daily for children (6-12 yrs)	11/2016-05/2018	24
Kudos Colostrum	capsules(n=60)	350 mg 100% bovine colostrum (min. 15-20% immunoglobulin antibodies and min. 0.4% lactoferrin) and gelatin (capsule shell)	Kudos Laboratories India, India, Hariyana, Faridabad, Faridabad	Internet	immunity strengthening	2× 1-2 capsules daily for adults, daily 1× 1 capsule for children on an empty stomach with a glass of water	01/2016-01/2019	21

Gotu Kola (Asiatic pennywort)	capsules (n=60)	Centella (*Centella asiatica*) (500 mg/capsule)	Goodcare Pharma Pvt. Ltd., India, West Bengal, South 24-Parganas, Bishnupur-1, Bagi	store (Hungary)	Helps cerebration, improves memory, concentration capacity, mental functions, decreases fatigue and depressive mood. Sleep disorders.	2× 1-2 capsules daily after meals with water	11/2016-11/2019	30
Malla Sindoor	powder	high purity mercury, trace elements containing mercury (I) chloride, sulfur, arsenic(III) oxide	Shree Baidyanath Ayurved Bhawan Pvt. Ltd. India, Maharashtra Vidarbha, Nagpur	Internet	malaria, cholera, syphilis, pneumonia, paralysis; asthma;	2× 65-125 mg daily with honey and ginger	08/2014-no expiry	2.5
Pama Malam	ointment	Content of 10 g: 500 mg *Gandhak* (sulfur); 250 mg *Haritala* (As_2S_3); 125 mg *Mansheela* (α-As_4S_4); 1 g *Shankajura* (talcum); 250 mg *Kattha* – Catechu tree *(Acacia catechu);* 250 mg *Karpura*-Camphorwood (*Cinnamomun Camphora*);	Amrut Pharmaceuticals, India, Karnataka Belgaum, Belagavi	Internet	scabies, eczema, ring-worm, psoriasis, barber's itch (*Pseudofolliculitis barbae), Tinea versicolor*	external use (before going to bed and shower, gently rub into the skin for a short time)	08/2016-09/2019	25

Qurs Kuhsta Qalai	tablets (n=60)	-	Hamdard Laboratories, India, Uttar Pradesh, Ghaziabad, Ghaziabad	Internet	disorders of male reproductive system (oligospermia, spermatorrhea, premature ejaculation, night pollution); antidepressant; testicle smooth muscle relaxant	2× 1 table daily with lukewarm milk	01/2016-01/2019	3.6
Swasari Ras (Lots 1 & 2)	powder	875 mg liquorice (*Glycyrrhiza glabra*); 500 mg clove (*Syzygium aromaticum*); 500 mg Ceylon cinnamon tree (*Cinnamomum zaylanicum*); 500 mg zebrawood (*Pistacia Integerrima*); 500 mg morning glory (*Cressa Cretica*); 375 mg ginger (*Zingiber officinale*); 375 mg black pepper (*Piper nigrum*); 375 mg long pepper (*Piper longum*); 250 mg calcinated mica ash [$K(Mg,Fe)_3(AlSi_3O_{10})(OH)_2$] (*Abrak bashma*); 250 mg ash of pearl oyster shell (*Muktashukti Bhasma*); 250 mg gypsum ash (*Godanti bhasma*); 250 mg cowry ash (*Kapardak bhasma*)	Divya Pharmacy, India, Uttarakhand, Haridwar Haridwar	Internet	asthma, cough, sinusitis, hay fever, headache	500-1000 mg 2-3×/day half an hour before meals or after with honey and hot water	01-2016/ 12-2020 & 07-2016/06-2021	10 & 40

| Zandu Shwas Kuthar Rasa | tablets (n=70) | high purity sulfur, aconite (*Aconitum ferox*), borax, realgar (α-As_4S_4); black pepper (*Piper nigrum*); dried ginger (*Zingiber officinale*); long pepper (*Piper longum*); 4.1 mg/tablet except for black pepper: 32.2 mg/tablet | Emami Ltd. India, Gujarat, Valsad, Vapi | Internet | asthma | 3× 1-4 tablets daily with water | 09/2013-08/2018 | 4.55 |

Table S2. Optimized microwave-assisted digestion steps applied for alternative medicines in PTFE vessels (n = 10) containing quartz microvials

Nº.	Time (min)	Nominal power (W)	Temperature limit (°C)
1	0-1	250	85
2	1-3	0	85
3	3-8	200	110
4	8-13	350	130
5	13-18	550	140
6	18-23	250	140
7	23-33	0	-

Table S3. Optimized operating conditions of the ICP-SF-MS measurements and monitored isotopes

Parameter	
RF power (W)	1200
Carrier gas (Ar) flow rate (L/min)	14
Outer gas (Ar) flow rate (L/min)	0.8
Nebulizer gas (Ar) flow rate (L/min)	1.1
Nebulizer	Meinhard (equipped with Scott spray chamber)
Sampler cone material and orifice diameter (mm)	Ni and 1.0
Skimmer cone material and orifice diameter (mm)	Ni and 0.8
Detection mode	peak jumping
Dwell time (s)	0.1
Detector	electron multiplier
Monitored isotopes	
Chromium	^{52}Cr
Manganese	^{55}Mn
Iron	^{56}Fe
Nickel	^{58}Ni
Copper	^{63}Cu
Zinc	^{66}Zn
Arsenic	^{75}As
Cadmium	^{111}Cd
Tin	^{120}Sn
Barium	^{138}Ba
Mercury	^{202}Hg
Lead	^{206}Pb + ^{207}Pb + ^{208}Pb
Germanium as internal standard (IS) for As	^{74}Ge
Indium as IS for the rest of elements	^{115}In
Resolution (R)	
low (for Cd, In, Sn, Ba, Hg, Pb) / high (for Ge, As)	R = 300 / R = 10000
medium (Cr, Mn, Fe, Ni, Cu, Zn, In)	R = 4000