



Analytical methodology

First comprehensive study on total contents and hot water extractable fraction of selected elements in 19 medicinal plants from various locations in Nyamira County, Kenya



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ABSTRACT

A large number of medicinal plants is traditionally known in Kenya and used for treatment of various diseases, for example diabetes, where metals are supposed to be involved in pathogenesis and therapy. Therefore, detailed investigation of the concentration of a large number of metals in medicinal plants is required for improved understanding and optimisation of the therapeutic role of metals and also to exclude potentially toxic effects. Our study focused on the determination of 30 selected elements in 19 medicinal plant species each collected from 3 sampling locations in Nyamira County, Kenya. The obtained comprehensive data set showed large variability and multivariate data analysis revealed that the differences in the elemental composition were stronger dependent on the plant species than on the sampling location. In addition, hot water extractions were performed to mimic the traditional preparation of medicine from the plants. It was found that the mean extraction efficiencies were below 20% except for B, Mg, P, K, Mn, Co, Ni, Cu, Zn, Rb, Mo, Cd and Tl, which are mostly essential elements apart from Cd and Tl. Sequential (ultra)filtration of the extracts was applied as novel approach for molecular size-fractionation of the extracted elemental species. The results indicate more than 50% low molecular weight species (<3 kDa) for Mg, Mn, Co, Ni and Zn while predominantly larger size-fractions (>3 kDa up to <5 μm) were detected for V, Cu, Al and Fe.

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1. Introduction

Traditional medicinal plants are an important part of health care in Kenya due to the large variety of more than 1200 freely and locally available wildlife plants with known therapeutic effects [1,2]. The high demand due to growing popularity accompanied by increased commercialisation of herbal medicine causes concerns about over-exploitation of the natural plant resources in Kenya [3,4]. Many studies focus on the documentation of the classification, identification and basic traditional knowledge on the preparation and medical application of most commonly used plants. For example, there are many medicinal plants with known anti-diabetic effects which offer the potential to provide affordable health care

to the rising number of diabetes patients in Kenya [5]. However, few studies focussed on detailed chemical analysis of the applied medicinal plants. In particular elemental concentrations can be relevant either for therapeutic use in case of deficiency or regarding potentially toxic effects in case of contamination of the plants. More specifically, sufficient evidence was found indicating that metals play an important role in development and treatment of diabetes [6].

Determination of selected metals was reported by Maobe et al. in 8 medicinal plants from Kisii region, Kenya concluding that the observed levels were within the allowed limits of WHO [7], by Adongo et al. in 7 medicinal plants from Chuka community, Kenya, suggesting potential therapeutic effects of Zn, Mg and Fe [8] and by Piero et al. in 5 antidiabetic medicinal plants from Kenya indicating that the determined levels of some elements with glucose lowering effects are sufficient to potentially contribute to the treatment of diabetes [9]. Oyaro et al. analysed metal contents in 6 medicinal

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plants from Narok County, Kenya, and also in hot water infusions of these plants [10]. It was found that the percentage extraction efficiencies of the investigated metals in the infusions were very low for Cu and Fe, but much higher for Cr and Co. The determined levels were considered safe for application due to the relatively low solubility in infusions.

All these studies applied atomic absorption spectrometry for elemental detection and thus a maximum of 10 elements was determined due to limited or lacking multi-element capability of this analytical technique. In addition the number of investigated plants was relatively low (5–8) due to the use of time-consuming wet ashing procedures. Samples were mainly collected from one location, in some cases from several locations, but finally mixed prior to the analysis and thus resulting in one average metal content per plant species. Variations of metal concentrations for the same plant species collected from different sites were not reported [7–10]. Considering that many medicinal plants are prepared by soaking in water, as decoction or as infusion prior to application, the extraction efficiency is an important parameter to estimate the actual dose of metals during therapy and needs more detailed investigation. Finally, there has been no attempt in the reported studies to characterise the elemental binding forms (species) in the plant extracts regarding their molecular size or structure. However, this information is required in order to better understand the potential role of metals in the therapeutic effect of the medicinal plants. Are certain metal species acting as therapeutic agents or is only the total metal content significant independent of the present elemental species? In analogy this also applies for potentially toxic effects. Considering the availability of the same species of naturally grown medicinal plants in various regions of Kenya, another important aspect is the variability of the elemental contents of the same plant species sampled from different locations.

The present study is the result of a co-operation between the Universities of Chuka and Nairobi, Kenya and the research center in Juelich, Germany. Joint resources allowed access to 19 medicinal plants from 3 sampling locations in Nyamira County, Kenya and determination of 30 elements using microwave digestion with subsequent quantification by inductively coupled plasma mass spectrometry (ICP-MS). Hot water extractions simulating the traditional preparation were performed for the plants from one location and analysed for the same elements in order to estimate the percentage of the total elemental content which is actually administered to the patient during therapy. Moreover, sequential (ultra-) filtration was applied for selected plant extracts as novel approach to achieve a quick size fractionation of the water soluble elemental species.

2. Experimental

2.1. Sample collection and pre-treatment

Samples from 19 medicinal plants were collected by herbalists in each of the following 3 districts of Nyamira County: Nyamira (NMP), Manga (MMP) and Borabu (BMP). The plants were identified by experts from the National Museum of Kenya, Nairobi. An overview of the investigated plants is given in Table 1 including the number coding for reference throughout this manuscript, e.g. NMP1 refers to *Warburgia ugandensis* from sampling location NMP. The collected plant samples were washed with deionised water to remove soil and other material from the plant surface, then air dried in the shade and pre-ground in a wooden mortar. Then, the samples (except for NMP3 and NMP17) were packed in polyethylene bags and shipped to research center Jülich for further analysis. The plant samples were fine ground using a ball mill with zirconium oxide vessels and balls (Pulverisette 6, Fritsch, Germany). Moisture was

determined in duplicate for approximately 0.5 g aliquots of ground plant material at 105 °C until constant weight.

2.2. Chemicals and reference materials

Nitric acid (65%, suprapure) and hydrogen peroxide (30%, suprapure) were obtained from VWR, Darmstadt Germany. Deionised water was prepared with a Millipore system. Reference materials NIST 1640a (natural water), NIST 1515 (apple leaves) and NIST 1547 (peach leaves) were purchased from LGC standards, Germany.

2.3. Microwave digestion

Aliquots of approximately 70 mg of the plant samples were digested in duplicate using 2 mL of nitric acid and 1 mL of hydrogen peroxide in a MARS 5 closed vessel microwave system (CEM, Germany) at 160 °C. Complete digestion of the organic matrix was achieved with occasionally slight silicate residues, which was fit for the purpose of this study. The digestion solution was transferred to calibrated polystyrene sample vials and made up to 10 mL with deionised water. Blank digestions and digestions of plant reference materials were processed in the same way.

2.4. Hot water extraction

Approximately 150 mg of ground plant material were mixed with 40 mL deionised water in a glass beaker including a glass rod to prevent retardation of boiling and covered by a watch glass. The sample suspension was heated within 6 ± 0.5 min to boiling temperature and kept boiling for 5.5 ± 0.5 min. Temperature was checked using a liquid-in-glass thermometer in a separate beaker containing water only. After cooling to room temperature the mixture was transferred to a polypropylene tube and shaken for 13 h in the dark on a horizontal shaker at 100 motions per minute. Loss of water due to evaporation was compensated by topping up with deionised water to 40 mL. Finally, the plant suspensions were filtered through 0.45 μm syringe filters to obtain clear extracts for elemental analysis.

2.5. Sequential (ultra-)filtration

Hot water extractions were prepared for BMP10 and NMP18 as described above ($n = 3$). In addition extractions of the same plant samples were performed by shaking with deionised water at room temperature in the dark for 13 h without any heating ($n = 3$). The obtained raw extracts were first filtered using a 5 μm syringe filter. An aliquot of the obtained filtrates was then filtered through 0.45 μm syringe filters. An aliquot of this second filtrate was subjected to ultrafiltration through 10 kDa membrane using Amicon filtration units at a speed of 14000 g (Merck-Millipore, Germany). An aliquot of the third filtrate was finally subjected to ultrafiltration through 3 kDa membrane using Amicon filtration units at a speed of 14000 g (Merck-Millipore, Germany). The ultrafiltration units were pre-cleaned by filtration of 0.5% nitric acid and deionised water prior to filtration of the samples following previous work [11]. The filtrates were analysed by ICP-MS as described below. The elemental contents were related to the solid plant material. In addition to the <3 kDa fraction, the size fractions 3 kDa–10 kDa, 10 kDa–0.45 μm and 0.45 μm –5 μm were calculated as difference of the contents in the respective filtrates.

2.6. Quantification by ICP-MS

Total contents of 30 elements (B, Mg, Al, P, K, Ca, Ti, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, Ga, Rb, Sr, Y, Nb, Mo, Cd, Ba, Nd, Sm, Gd, Er, Yb, Tl and Pb) were determined in the digestion solutions of the plant

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