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Effect of Farm Additives on the Potential Bioavailability of Some Nutritional Elements from Kenyan Wild Plants

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Abstract
In this study, the effects of farm additives on eight wild plants from Nyamira County, Kenya were evaluated for their release of iron, copper, calcium, potassium and magnesium. A hundred and sixty traditional medicinal practitioners were surveyed and found to use *Solanum indicum*, *Carissa edulis*, *Urtica dioica*, *Clerodendrum myricoides*, *Aloe vera*, *Plectranthus barbatus*, *Bidens pilosa* and *Solanum mauense*. Atomic absorption spectrophotometer was used to determine the total nutritional element contents in the plants while ultra filtration and physiologically based extraction tests were used to determine the release and solubility of the nutritional elements. The plants from areas with high use of farm additives were found to have statistically significant high total levels of copper from the area with no or little application. Elemental analysis of the molecular species fractions into < 3 kDa, 3–10 kDa, 10 kDa–0.45 μm and 0.45–5 μm mass fractions showed that the mass distribution of the elements in the plants depended on the element. The nutritional elements released by gastrointestinal digestion were more than those released aquatically. Farm additives had no significant effect on the levels of most nutritional elements determined and the plants can be used as mineral element supplements in the human body in addition to their therapeutic activity.

Keywords Agricultural activities · Anaemia · Bioavailability · Fractionation · Nyamira

Introduction

Bioavailability of elements in plants depends on various parameters that include soil type, rainfall and the prevailing environmental growing conditions that are heavily influenced by anthropogenic activities in the area. Iron, potassium, magnesium, calcium and copper are among the elements useful and essential in the human body that are found in plants. Commercial tea and coffee production require the use of fertilizers and other additives for high productivity [1]. Minimum concentration of nutrient required for fertilization of crops depends on type of crop, type of nutrients, soil conditions and composition, cropping season, and plant growth stage. Use of excessive of fertilizers in most cases leads to increase in soil acidity or salinity making plants grown in those areas to be toxic [2] and unbalanced fertilizer application leads to negative effects on crop physiology and production [3]. Chemical fertilizers replenish the depleted nutrients in optimum quantities to sustain the soil fertility [4]. Most fertilizers and other additives used in Kenya are imported. For example, the most popular tea formulation fertilizer imported is the NPK 25: 5: 5 or NPK 20: 10: 10 [5]. Most of the fertilizers are imported from far countries making them not to be readily available. Hence, some companies have begun the production of blended fertilizers in Kenya to address the fertilizer shortage. The possible negative impacts of the locally manufactured fertilizers and mitigation measures have not been established leave alone the elucidation of the actual nutrient composition. The soil nutrients availability, their content and degree of accessibility are dynamic depending on inorganic and biochemical processes in the soil [6] and biotic and abiotic factors such as temperature, water content, soil reaction, and nutrient uptake, input, and losses [7] affect the release of a specific nutrient element from the soil to the plants (those grown on the

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agricultural fields and the wild plants which grow adjacent to the fields). Although essential for achieving high crop production excessive application of farm additives could contribute to environmental pollution which poses substantial risks to human health [8–11]. The nutritional elements have various functions and are required by the human body in different quantities. Iron is majorly used in the synthesis of haemoglobin and myoglobin in the human body. Iron deficiency results in hypochromic anaemia [12, 13] while prolonged excessive iron levels in the human body result in liver and heart damage, diabetes and skin diseases [14]. The number of people afflicted by anaemia in developing countries has been increasing over the years as supplementation programs have not succeeded and other health complicating factors such as malaria, haemoglobinopathies and parasitic gastrointestinal infections are on the increase [14–16].

Magnesium acts as Ca antagonist on vascular smooth muscle tone and post-receptor insulin signaling. It is involved in energy metabolism, release of neurotransmitters and endothelial cells, and a cofactor of about 300 enzymes [13, 17]. Mg deficiency is related to aging and age-related disorders such as diabetes, metabolic syndrome, hypertension and several cardiovascular conditions [18, 19].

Calcium is a component of the bones and teeth; it plays a vital role in blood clotting as well as in the management of cancer [18]. Calcium in the human body participates in biological function of musculoskeletal, nervous and cardiac systems and parathyroid. It acts as a cofactor in enzyme reactions and in the maintenance of mineral homeostasis [20–23]. Calcium intake moderately has been shown to reduce the risk of colon cancer [24].

Potassium is used in the homeostatic regulation of osmotic pressure, protein and carbohydrate metabolism [25]. K is involved in the maintenance of balance of the physical fluid system, transmission of nerve impulses and heart activity of muscle contraction [26–28]. K deficiency results in fatigue, cramping legs, muscle weakness, slow reflexes, acne, dry skin and irregular heart beat and it may produce alkalosis which makes the kidney unable to retain K. Excess K is associated with hyperkalaemia which leads to kidney failure [27] while copper is involved in enzyme function, development of connective and nerve coverings (myelin sheath) and in iron metabolism [12, 17]. Cu deficiency causes normocytic, hypochromic anaemia, leucopoenia, neuropenia and skeletal disturbances [12]. Excess levels of copper in the body are related to liver damage and gastrointestinal effects with cramps, nausea, diarrhoea and vomiting [28]. The wild plants which are used for therapeutic and nutrient elements supplementation should have the right quantity of the elements to enhance the beneficiation and to avoid toxicity.

Analyzed water soluble forms of nutritional elements in infusions of leaves, roots and fruits of the plants have shown that the extracted amount is a small percentage [29–32]. The presence of other nutrients such as vitamin C, organic acids, zinc or phytates (e.g. citric, lactic or malic acid) in the diet can increase the absorption of nutritional elements [32, 33]. Copper, calcium, phosphorus, potassium, sulphur and nitrogen are the main elements used in the manufacture agricultural additives in the third world countries such as Kenya. The use of these additives will elevate the levels of the nutritional elements in the soil and plants.

The aim of this study work was to investigate the effect of farm additives and plant species on the bioavailable contents of iron, copper, magnesium, potassium and calcium in eight plant species collected from Nyamira County, Kenya. The elemental levels in wild plants which are used therapeutically by man should be monitored to ascertain their efficacy and toxicity. Twenty-one medicinal plants from three sub-counties were screened for the total levels of iron, copper, magnesium, potassium and calcium of which eight plants which were found to have substantial amounts of the elements were selected and used in the study. The total levels of iron, copper, magnesium, potassium and calcium in the plants were determined by flame atomic absorption spectrometry (FAAS) to elucidate the effect of farm additives. The fractional species of the elements were determined by ultra filtration and in addition an enzymatic extraction procedure (EEP) was applied to determine the extent of bioaccessibility and the potential bioavailable fraction of iron, copper, magnesium, potassium and calcium to the human body [20, 32–34].

Methodology Experimental Procedures

Recruitment of Herbalists and Sampling of Plant Materials

Herbalists practicing in the three sub-counties of Nyamira County, Kenya were recruited depending on the use of various fertilizers and pesticides on their farms. The plants in Ekerenyo and Borabu were collected from around large-scale tea and coffee cultivation farms whereby pesticides, herbicides and fertilizers were applied heavily regularly while those from Nyamira were collected from areas with minimal or no use of farm additives. The protocols adopted in the study for preparation of the wild plants were based on the information obtained from the herbalists. The selected plant samples were taken to the Kenya national museum whereby they were identified and their voucher samples kept. Ten TMPs from each of the three sub-counties were recruited and requested to provide 1 kg of the dry plant sample of each plant species. The plant samples collected were washed with deionized water, air-dried under shade, placed in paper envelope, sealed and transported to the laboratory. Each sample was placed in a clean paper envelope and stored in a cool dry place until analysis. The samples were grinded using pestle and mortar to fine powder, placed in clean paper envelopes, labeled and stored in cool dark lockers.

This was done with ultimate care to ensure that there was no contamination during collection, transportation and storage of the plant samples. The plants used in this study were *Aloe Vera*, *Carissa edulis*, *Plectranthus barbatus*, *Urtica dioica*, *Bidens pilosa*, *Solanum indicum*, *Solanum mauense* and *Clerodendrum myricoides*.

Reagents and Chemicals

All the reagents used in the study were of analytical grade. Calibration standards were prepared from Certipur stock solutions (Merck, Darmstadt, Germany) in 3% nitric acid. Deionized 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.

Determination of Total Nutritional Elements by FAAS

The determination of the total iron, copper, calcium, magnesium, and potassium in the wild plants was carried out as described in Mogwasi et al. [32]. Homogenized 1.0 g of powdered plant sample was accurately weighed into a 100-mL Pyrex beaker which had been washed with 0.1 M HNO_3 and rinsed with distilled water. Aliquots of 4 mL of perchloric acid, 25 mL concentrated HNO_3 and 2 mL concentrated H_2SO_4 were added. The mixture was initially heated at 60 °C for 30 min, and then on a hot plate under an acid fumehood, until dense white fumes appeared. The heating was continued for 2 min afterward to remove the acid fumes which would otherwise corrode the FAAS metallic parts during the analysis. An aliquot of 50 cm^3 of distilled water was added on cooling to room temperature and boiled further for a half minute. The solution was allowed to cool and filtered using Whatman No. 42 filter paper into a 100-mL volumetric flask. Distilled water was added to the mark and the filtrate analyzed for iron, copper, magnesium, potassium and calcium using FAAS (210 VGP Flame Atomic Absorption Spectrophotometer). Each sample was analyzed in triplicate ($n = 3$) to test for homogeneity and analytical reproducibility.

Sequential Ultra Filtration for Determination of Nutritional Elements in Plants

The sequential ultra filtration of elements in plant samples was carried according to the procedures described in the Nischwitz et al. technique [31]. One hundred and forty milligrams (140 mg) of ground plant material were mixed with 40 mL deionized water in a polypropylene tube and shaken for 13 h in the dark on a horizontal shaker at 100 motions per minute ($n = 3$). Loss of water due to evaporation was compensated by topping up with deionized water to 40 mL. The obtained extracts were first filtered using a 5-μm syringe filter. An aliquot of 15 mL of the obtained filtrates was then filtered through 0.45-μm syringe filters. An aliquot of 10 mL of the second filtrate was subjected to ultra filtration through 10 kDa membrane using Amicon filtration units at a speed of 14,000*g* (Merck-Millipore, Germany). An aliquot of 5 mL of the third filtrate was finally subjected to ultra filtration through 3 kDa membrane using Amicon filtration units at a speed of 14,000*g* (Merck-Millipore, Germany). The ultra filtration units were pre-cleaned by filtration of 0.5% nitric acid and deionized water prior to filtration of the samples following previous work.

The water extracts and filtrates obtained from sequential filtration were analyzed using FAAS. NIST 1640a natural water reference material and NIST 1547 peach plant reference material were analyzed for quality control.

Physiologically Based Extractions of Mineral Elements from the Wild Plants

The in vitro gastrointestinal digestion method used was based on Intawongse and Dean [20]. An accurately weighed plant material (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 deionized water and the pH adjusted to 2.5 with concentrated hydrochloric acid). The mixture was shaken at 100 rpm in a thermostatic bath maintained at 37 °C for 1 h. After 1 h, the solution was centrifuged at 3000 rpm for 10 min and 5 mL aliquot was removed and filtered through a 0.45-μm micro filter and replaced with the original gastric acid solution to retain the original solid to solution ratio.

In order to create the small intestinal digestion conditions, 52.5 mg bile salts and 15 mg of pancreatin were added into the same sample tube and saturated sodium bicarbonate solution was added to set the pH to 7.0. The mixture was then shaken at 100 rpm in a thermostatic bath 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 reached. The remaining solution was centrifuged at 3000 rpm for 10 min and the residue was retained for further acid microwave digestion and FAAS analysis. All aliquots were stored at 4 °C and analyzed within 24 h with FAAS. Before analysis, the sample was diluted 1:10 w/w. Gastric, intestinal and water extracts were performed in every batch and all the samples were extracted and analyzed in triplicate. The bioaccessible metal contents of plant extracts (gastric and intestinal fractions) were determined by FAAS.

Results and Discussion

Total Nutritional Element Contents in Wild Plants from Three Sampling Locations

The total concentrations of the nutritional elements in the wild plants from the three sub-counties with different agricultural additives from Nyamira County determined by FAAS are presented in Table 1 including the minimum and maximum means for the areas. The range of the mean contents of the same element in the various plants from the same sampling location is often broad with maximum concentrations approximately 3-fold to 10-fold higher than the minimum concentration. In a few cases, large span of more than 10-fold were observed: 12- and 13-fold for calcium in Borabu and Ekerenyo wild plants and 12-fold for iron in Nyamira wild plants. The levels of Ca, Mg, K and Fe in the wild plants did not depend on the collection area while that of copper was lowest in Nyamira wild plants.

Fertilizers are used to improve the quality and quantity of leafy vegetables and nutritional status of plants. Some of the fertilizers used in Kenya include triple supper phosphate and calcium ammonium nitrates (CAN) which are calcium based used in maize and vegetable production, potassium nitrogen phosphate (NPK) and DGHP fortified with trace amounts of calcium, iron, magnesium and copper used in the tea and coffee production [1–5]. In addition, lime is applied to neutralize the soil acidity after application of fertilizers for a period of five years [35]. The amount of calcium, potassium, iron, magnesium and copper in the plants from Ekerenyo and Borabu was expected to be significantly high than those from Nyamira. But calcium, potassium, iron and magnesium levels in the wild plants from Nyamira were not statistically different from those from Borabu and Ekerenyo ($P < 0.05$) (Table S2). This could have been due the lower calcium, potassium, iron and magnesium absorption influenced by the rock type from which the soil was formed and the ability of the plant species to accumulate the elements. The actual composition of calcium, potassium, iron and magnesium in the additive could be lower than what was documented. The results reported in the present study are similar to those reported by Grimm-Wetzel and Schonherr (2007) that Ca application increases the Ca content in peripheral layers of apple fruits and reduced the K concentration [36]. Val et al. (2008) reported that the fruit skin and several fertilizer applications were needed to promote a prolonged increase in the Ca concentration in the skin [37] and Ca applications did not influence the Mg and K concentrations while it has been reported that the application of $CaCl_2$

Table 1 The mean total levels plus standard deviation of Mg, K, Ca, Fe and Cu in wild plants from Borabu, Ekerenyo and Nyamira

Wild plant	Study area	Mg (g/kg)	K (g/kg)	Ca (g/kg)	Fe (mg/kg)	Cu (mg/kg)
S. indicum	Borabu	0.67 ± 0.1	12.00 ± 2.0	3.14 ± 0.07	464.00 ± 2.37	9.72 ± 0.21
	Ekerenyo	0.64 ± 0.1	12.01 ± 2.0	3.11 ± 0.07	453 ± 2.3	8.7 ± 0.36
	Nyamira	0.94 ± 0.2	9.50 ± 0.50	5.30 ± 0.06	175.76 ± 5.03	3.73 ± 0.25
P. barbatus	Borabu	4.91 ± 0.1	33.00 ± 2.0	20.40 ± 0.4	2000 ± 70	14.70 ± 0.20
	Ekerenyo	4.71 ± 0.1	33.03 ± 2.0	20.40 ± 0.4	2010 ± 70	13.70 ± 0.20
	Nyamira	4.30 ± 0.2	40.00 ± 2.0	13.40 ± 0.5	1240 ± 50	4.60 ± 0.40
U. dioica	Borabu	6.20 ± 0.4	36.00 ± 6.0	39.00 ± 1.0	1004.30 ± 7.8	15.94 ± 1.5
	Ekerenyo	6.23 ± 0.4	36.04 ± 6.1	39.90 ± 0.80	923 ± 4.33	14.2 ± 1.24
	Nyamira	5.50 ± 0.2	29.00 ± 2.0	39.00 ± 1.0	1756.36 ± 8.95	3.88 ± 0.15
B. pilosa	Borabu	3.80 ± 0.2	26.00 ± 4.0	11.90 ± 0.3	1184.37 ± 0.38	7.76 ± 1.5
	Ekerenyo	3.83 ± 0.2	26.02 ± 4.0	11.94 ± 0.3	1207 ± 3.33	8.1 ± 1.23
	Nyamira	4.70 ± 0.2	37.00 ± 2.0	13.50 ± 0.3	1582.39 ± 5.05	4.82 ± 0.23
S. mauense	Borabu	1.88 ± 0.1	46.00 ± 3.0	9.90 ± 0.60	985.76 ± 6.20	15.53 ± 0.2
	Ekerenyo	1.83 ± 0.1	46.01 ± 3.2	9.92 ± 0.62	983 ± 3.34	15.2 ± 0.53
	Nyamira	2.60 ± 0.2	35.00 ± 5.0	9.20 ± 0.20	978.73 ± 8.3	3.21 ± 0.03
C. myricoides	Borabu	2.51 ± 0.3	20.00 ± 2.0	12.50 ± 0.7	1197.02 ± 14	12.25 ± 1.5
	Ekerenyo	2.61 ± 0.1	20.03 ± 2.0	12.51 ± 0.7	1242 ± 5.83	13.5 ± 0.33
	Nyamira	1.25 ± 0.1	7.80 ± 0.30	12.50 ± 0.1	985.40 ± 5.06	3.61 ± 0.16
C. edulis	Borabu	1.41 ± 0.1	9.80 ± 0.40	24.90 ± 0.7	2635.48 ± 17.33	7.13 ± 1.4
	Ekerenyo	1.44 ± 0.1	9.83 ± 0.40	24.93 ± 0.7	2529 ± 6.76	8.1 ± 0.48
	Nyamira	0.67 ± 0.1	9.70 ± 0.20	10.10 ± 0.2	2143.23 ± 9.3	2.12 ± 0.02
A. vera	Borabu	4.20 ± 0.5	47.00 ± 5.0	32.00 ± 3.0	1243.22 ± 9.41	6.2 ± 0.63
	Ekerenyo	4.24 ± 0.5	47.02 ± 5.0	32.04 ± 3.0	1303 ± 4.52	7.99 ± 0.32
	Nyamira	5.26 ± 0.1	59.00 ± 7.0	34.00 ± 1.00	1313.59 ± 3.72	3.88 ± 0.91

increased Ca content in litchi fruit increasing the firmness and skin colour and showed a positive correlation with leaf and fruit K [36, 37]. This means that different plants absorb and accumulate different amounts of calcium, iron, potassium and magnesium in their tissues.

The fungicides used in Kenya contain copper as one of the main ingredients in the form of copper oxide (CuO), copper oxychloride, copper ammonium acetate and copper hydroxide while copper sulphate ($CuSO_4.5H_2O$), copper chloride ($CuCl_2$) and sodium copper chelate ($Na_2CuEDTA$) are used in foliar fertilizers for tea and coffee [1, 38]. The use of the farm additives raised the copper levels in the soil and the plants. Ekerenyo and Borabu sub-counties are large-scale tea and coffee producers with regular use of fungicides and foliar fertilizers (usually 75 kg/hectare per year) [39]. This accounted for the difference in the copper levels in the present study compared with that reported in our previous study [32]. The values for the *t* tests for copper determined for plants from Borabu and Nyamira, Ekerenyo and Nyamira and Borabu and Ekerenyo were 3.744, 3.326 and 0.337 respectively. This shows that there was a significant difference in the total levels of copper between plants collected from Borabu and Nyamira and Ekerenyo and Nyamira ($P < 0.05$) (Table S2). This means that the use of agricultural additives influenced the copper levels in the plants. The levels of copper in the plants in the present study are lower than those reported by Anna et al. of 9.40–16.70 mg/kg among the Brazilian teas [30] and Adedapo et al. of 240 mg/kg in the South African medicinal plants [16] due to the influence of the farm additives in our study.

From the results for the investigated sampling locations in the three sub-counties from Nyamira County, it can be concluded that the environmental conditions of the location (soil, water, air pollution potential influence from agricultural activities) had minor influence on the Fe, Mg, Ca and K but the agricultural activities of the location influenced the copper levels in the wild plants used therapeutically. Much of the influence on the nutritional element content was the plant species. This is due to the different sizes of the investigated plants (herbs and trees) which reached different soil horizons and thus different sources of metals with their roots. Also different parts of the plants were sampled in different plant species (roots, stem bark and leaves) as they were the ones used for medical purposes.

Some of the plants investigated were used in the management of anaemia. In this case, Fe levels in the plants are of special interest, as it is known to support the synthesis of the red blood cells boosting the blood levels and hence alleviating the anaemia condition [38]. The soils of Nyamira County are generally ferruginous, with relatively high cation exchange capacity meaning that the plants could absorb high levels of iron from the soil as depicted from the results obtained in the study. The levels of iron obtained in our study are comparable with those of Adedapo et al. of 986 mg/kg in *B. pilosa* and 2550 mg/kg in *C. album* in South Africa [16]. Alikwe et al. also reported iron levels of 789 ± 0.1 mg/kg in *B. pilosa* from Nigeria [40] and Majolagbe et al. (2013) in some selected blood-building medicinal plants in Southwest Nigeria [38].

The wild plants are also used in the treatment of diabetes. In this regard, the levels of magnesium in the plants is of special interest as it is known to be one of the hypoglycemic elements. The levels of magnesium reported in the present study are similar to those reported by Adongo et al. working on five Kenyan antidiabetic medicinal plants traditionally used to manage diabetes mellitus of 1021.4 ± 6.3 mg/kg in *Erythrina abyssinica*, 791.7 ± 1.2 mg/kg in *Catha edulis*, 865.5 ± 2.8 mg/kg in *Strychnos henningsii*, 823.6 ± 2.3 mg/kg in *Aspilia pluriseta* and 879.7 ± 3.1 mg/kg in *Bidens pilosa* [41] and those of Mussie et al. of 0.545 to 73.415 g/kg in antidiabetic medicinal plants from Eritrea [36], and potassium levels reported by Magili et al. of 0.7 to 35.88 g/kg in selected antidiabetic medicinal plant parts used in Adamawa state, Nigeria [42] and to those reported by Mussie et al. of 3.388 to 10.6556 g/kg among selected antidiabetic medicinal plants from Eritrea [43].

This means that the use of farm additives had minimal influence on the nutritional elements (Ca, Fe, K and Mg) and the wild plants can safely be used for the therapeutic purposes the locals put them into in addition to the use for nutritional elements supplementation. Most of the fertilizers used in Kenya contain K, Fe, Mg and Ca while the pesticides contain copper [39, 44]. The frequency of the application of these farm additives will affect the amount of these elements in the soil and hence their availability to the plants. This requires a controlled study on the frequency of farm additives application on the farms while monitoring the levels of the nutritional elements in plants over a given period of time to come up with the exact effect of the farm additives on the levels of nutritional elements.

Ultra Filtration of Mineral Elements in the Wild Plants

The cold water extracts of wild plants from Borabu were subjected to a 4-step sequential filtration procedure resulting in the following size fractions as described in the experimental section: < 3 kDa, 3–10 kDa, 10 kDa–0.45 μm and 0.45–5 μm. Elemental analysis of the fractions clearly showed that the mass distribution across these fractions was dependent on the element and the plant species. The results for calcium, copper, iron, potassium and magnesium are summarized in Table S1 and Figs. 1, 2 and 3 and Figs S1-S2.

In *S. indicum*, *P. barbatus*, *U. dioica*, *B. pilosa*, *S. mauense* and *C. edulis*, the 10 kDa–0.45 μm molecular mass iron species were predominant while in *C. myricoides* and *A. vera*, the 0.45–5 μm molecular mass species were of highest occurrence. The 3–10 kDa molecular mass species were of least

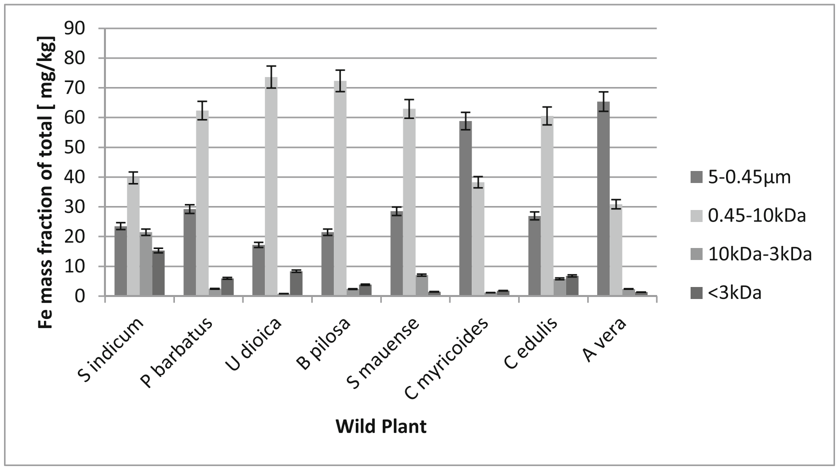

Fig. 1 Percentage distribution of Fe across size fractions obtained from sequential filtration of aqueous extracts of selected wild plants

occurrence in most plants except in *S. indicum*, *S. mauense* and *A. vera* in which the < 3 kDa molecular mass species were of lowest occurrence. Iron in most plants occurred in > 0.45 µm molecular size mass species (Fig. 1). Iron in the wild plants existed mainly in moderate large molecular mass species (10 kDa–0.45 µm) and this means that when the plants are consumed, the Fe species present could not easily diffuse through the cell membranes and hence alternative mechanisms such as active transport could be used to avail it to the body tissues [45]. The extraction efficiency of iron in *S. indicum* in the present study was relatively higher than that reported in most studies. This could be due to the ligands present in the plant bound weakly to iron which make it to be released easily under the reaction conditions and this needs to be investigated further to elucidate the specific ligands in the plant species.

In *C. myricoides*, *C. edulis* and *A. vera*, the 10 kDa–0.45 µm molecular mass species of copper were of highest occurrence and 0.45–5 µm molecular mass species were of lowest occurrence in *C. myricoides* and absent in *C. edulis* and *A. vera* (Fig. 2). In *S. indicum*, *P. barbatus*, *U. dioica*, *B. pilosa*, and *S. mauense*, the smallest molecular mass species (< 3 kDa) were predominant while the largest molecular mass species were of lowest occurrence in the species (0.45–5 µm) except in *P. barbatus* where 3–10 kDa molecular mass species were of lowest occurrence as we had reported in our previous study [34]. Copper compounds used in farm additives such as pesticides are in small complexes. When these are absorbed by plants, they form low molecular mass species compounds in the wild plants. The copper compounds in the farm inputs influence the absorption of the copper. This means that the ligands complexed with copper in the plants strongly which influenced the extent of their release and their bioavailability to the body [46].

In *P. barbatus*, *B. pilosa* and *C. myricoides*, the 10 kDa–0.45 µm molecular mass species of Ca were of highest occurrence followed by < 3 kDa molecular mass species. In *S. indicum* and *S. mauense*, the < 3 kDa molecular mass

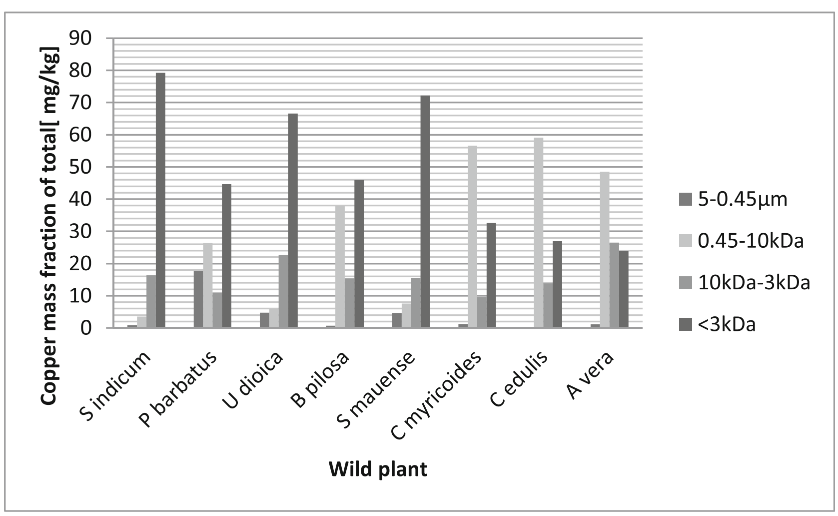

Fig. 2 Percentage distribution of Cu across size fractions obtained from sequential filtration of aqueous extracts of selected wild plants

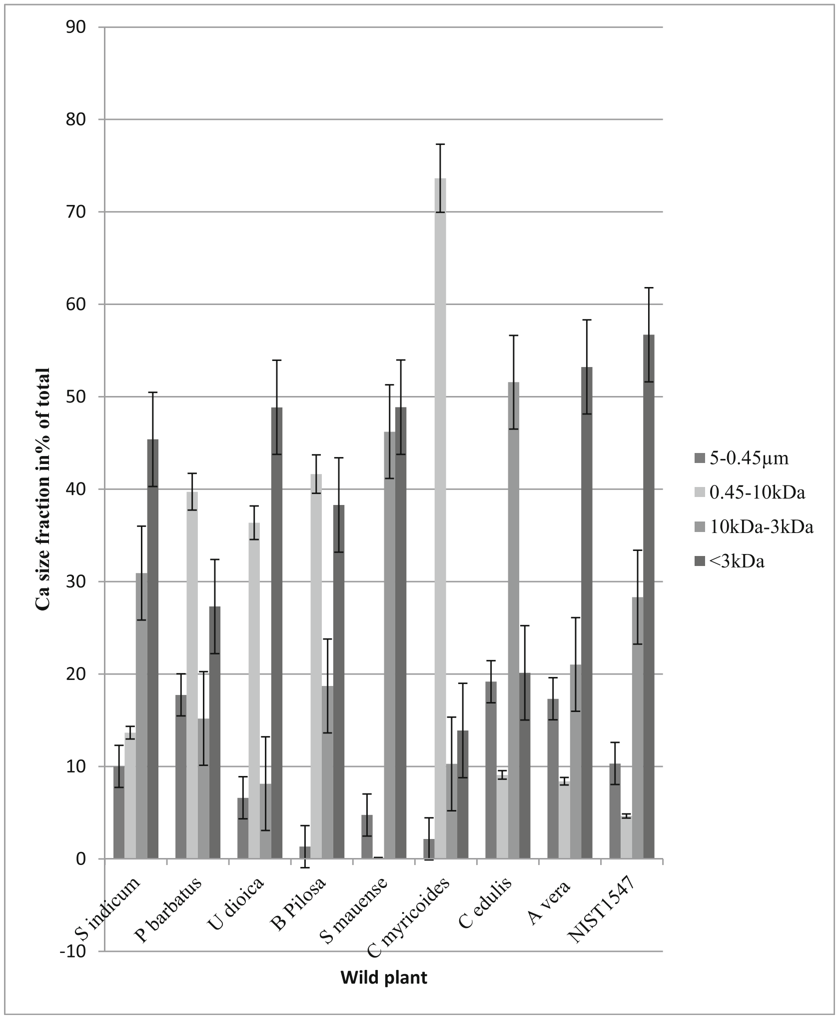

Fig. 3 Percentage distribution of Ca across size fractions obtained from sequential filtration of aqueous extracts of selected wild plants

species of Ca were of highest occurrence while in *C. edulis*, the 3–10 kDa molecular mass species were of highest occurrence (Fig. 3). In most plants, the large molecular mass species (> 0.45 μm) were of least occurrence. Calcium in the wild plants existed in < 10 kDa molecular size species which can easily diffuse through the cell membranes in the body and be availed to the body of the consumer of the plant. This means that the wild plants can be used as an alternative source for calcium supplementation in case of Ca deficiency.

Potassium mainly existed in moderate higher molecular masses in the plants investigated with 10 kDa–0.45 μm as the predominant molecular mass species except in *S. indicum* where < 3 kDa molecular species were of highest occurrence (Fig S1). The smallest molecular mass species were the lowest existence in the plants (< 3 kDa). Potassium in the wild plants could not easily diffuse through the cell membranes when the plants are consumed and hence alternative mechanisms such as active transport could be used to avail it to the body tissues.

Magnesium predominantly existed as large molecular mass species (> 10 kDa) in all the plants. *C. myricoides* and *C. edulis* had the largest molecular mass species (0.45–5 μm) as the predominant species while in the rest of the plants, the moderately molecular species (10 kDa–0.45 μm) were the predominant species (Fig S2). The highest concentration of magnesium of 3.3 g/kg was detected in 10 kDa–0.45 μm mass size fraction from *A. vera* while the lowest concentration of 25 mg/kg was in *S. indicum*. The lower molecular mass species of magnesium were absent in the plants. Magnesium in most wild plants existed in > 10 kDa molecular mass species which could not easily diffuse through the cell membranes in the body and alternative mechanism such active transport are required to avail it to the human body when the wild plants are consumed.

Different elements existed in different sizes in the wild plants. Mg, Fe, Cu and K existed as large molecular species while Ca existed in lower molecular species. This means different mechanisms are used for the uptake of different ions in the human body form the plants. Ca can diffuse easily across the cell membranes while the uptake of Fe, Mg, Cu and K can occur through the active transport to avail the nutrients to the body when the wild plants are consumed.

Physiologically Based Extractions of Mineral Elements from the Wild Plants

The wild plants from Borabu were subjected to physiologically based extractions of two-step enzymatic extraction according to Intawongse [20] and Mogwasi et al. [32] using the Borabu wild plants. The amount of each nutritional element extracted in each phase was divided by the total amount of the nutritional element extracted (extracted by acid digestion) to give the elemental percentage (%) extracted. The highest and lowest nutritional element gastric phase extraction percentage of 61.35% was from calcium in *C. myricoides* and 0.76% of potassium from *C. edulis* while the highest and lowest in the intestinal phase of 43.43% of magnesium and 0.74% of potassium were from *U. dioica* and *C. edulis* respectively. The highest and lowest total enzymatic nutritional element extraction were 98.43% of calcium from *U. dioica* and 1.5% of potassium from *C. edulis* while the highest and lowest aquatic extractions of 91.3 % of calcium and 0.18% of potassium from *U. dioica* and *C. myricoides* respectively (Figs. 4 and 5, Fig S3-S5). The extraction efficiency for each enzymatic phase and aquatic differed from one nutritional element to the other and is different in the wild plant species.

The percentage extraction of copper in the present study is different from what we had reported in similar plants [34]. This could be due to the ligands which complex with copper in the presence of farm additives residues favouring the degradation of the copper complexes in acidic media and hence making the extraction efficiency in the gastric phase to be high [46].

The physiologically based extractions assess the bioaccessibility of the nutritional element that is dissolved during the digestion process and is available for absorption into the circulatory system. The amount of most elements extracted in the intestinal phase was more than that extracted in the gastric phase. The in vitro determination of iron, copper, magnesium, calcium and potassium in the wild plants which was done to simulate the human digestion revealed that the amount of the nutritional element released enzymatically from the wild plants was more than that obtained by water extraction. This means that the potentially bioavailable form (PBF) of the nutritional element could be availed by the enzymatic break down in the human body [32]. These results are similar to those reported by Intagwonse and Dean [20] for the oral bioaccessibility of metals in vegetable plants grown in contaminated soil. Information about bioaccessibility of nutritional bioactive compounds such as mineral nutrients in wild plants is important to ascertain their nutritional efficacy in improving and maintaining human health [20, 25, 30, 47–52]. Most of the plants have a high potential of releasing the mineral nutrients investigated and can be used as supplements of the nutrients and are safe to be used.

Our results are contrary to those reported by Cadkova et al. which showed that the amount of iron and copper extracted in

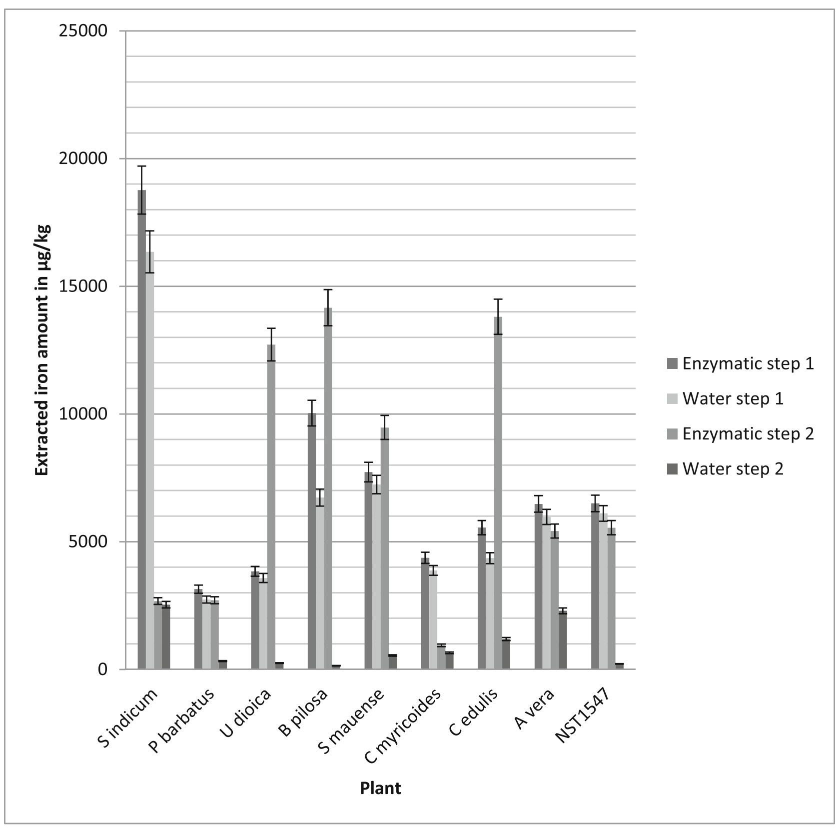

Fig. 4 Comparison of 2-step aqueous and enzymatic extraction for Fe in selected wild plants

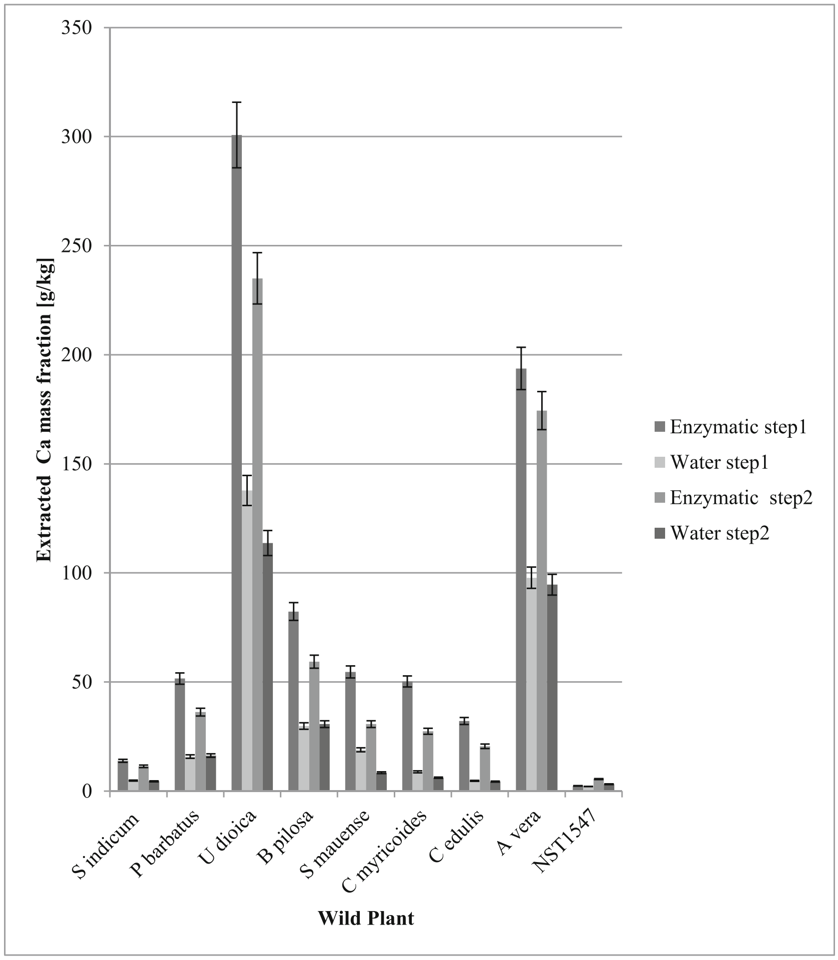

Fig. 5 Comparison of 2-step aqueous and enzymatic extraction for Ca in selected wild plants

the gastric phase was more than that extracted in the intestinal phase [46]. The copper complexes with organic ligands in the farm additive residues to form compounds which break down to different extend. The behaviour of iron differs slightly from that of copper as it had low bioaccessibility under simulated stomach conditions due to the high concentration of phytates and phytosterols which strongly bind iron in an acidic environment [53]. Iron bioaccessibility in artificial intestinal conditions is enhanced as iron forms soluble complexes with chelating ligands which are released in the intestinal juices during digestion. The copper in the plant facilitates the conversion of iron(II) into iron(III) which is used in the synthesis of erythrocytes in the human body and hence the blood synthesis making the plant to be used in managing anaemia [38, 54, 55].

The information on the forms (species) of the nutritional elements in the plant is essential in order to predict the impact of the metal on the human body. It measures the level of solubility and release of the metal from the plant. The bioavailable fractions of iron in these plants compared very well with those reported by Omolo et al. in medicinal plants from Kenya [54]. However, the Nigerian medicinal plants were reported to have higher bioavailable fractions of iron by Majolagbe et al. [38]. Li and Deng also pointed out that the bioavailable form of the metal obtained depended on the extraction solvent, plant part used and the plant species [52].

Conclusion

The performed strategy of total determination of total nutritional elements, ultra filtration and physiologically based extractions showed that the use of farm additives influenced the nutritional elements differently in wild therapeutic plants collected from three sub-counties in Kenya. This approach supports the pharmaceutical and toxicological investigations on the influence of farm additives on the nutritional elements in plants grown under different environmental conditions with different agricultural activities especially the uptake and metabolism of organic as well as metallic compounds. Based on the data obtained, the most suitable plants can be selected out of the many wild plant species in Kenya and other African countries for the control and safe therapy of diseases and conditions such as anaemia and diabetes as well as for the nutrient element supplementation. However, a controlled study on the application of farm additives on different plants over a period of time is required to elucidate the exact influence of the bioavailability of the nutritional elements. In addition, the most efficacious age of the plant, the organic ligands of calcium, copper, iron magnesium and potassium in the wild plants need to be elucidated for detailed organometallic and metalloprotein compounds determination in the wild plants using HPLC-electron spray ionization (ESI)-MS/MS.

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Compliance with ethical standards

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

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