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Bio-accessibility of iron and copper from seven Kenyan anti-anaemia medicinal plants

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Abstract: Anaemia is a blood disorder which is widespread among populations of the world. In this study, seven medicinal plants used by one hundred and sixty herbalists from Nyamira County to manage anaemia were evaluated for iron and copper bio-accessibility. The competence of four hundred herbalists was assessed, of which 160 participated in the study. Twenty-one different medicinal plants were reported to be used by various herbalists to manage a number of ailments afflicting their communities in three sub-counties in Kenya. A total of ten different medicinal plants were found to be used by the herbalists to manage anemia which is endemic in the country. Seven of the medicinal plants, *Aloe Vera, Carissa edulis, Croton macrostachyus, Clerodendrum myricoides, Melia azedrach, Toddalia asiatica and Warburgia ugandensis*, were studied by all herbalists. The seven medicinal plants were prepared according to the herbalists' procedure and analyzed for iron and copper through flame atomic absorption spectrophotometer. The mean iron level in *Carissa edulis* from Borabu was the highest (2635.48 ± 17.33 mg/kg), while that in *Warburgia ugandensis* from the Nyamira study area was the lowest (175.76 ± 5.03 mg/kg). The highest mean copper level (9.2 ± 1.24 mg/kg) was reported in *Melia azedrach* from Ekerenyo, while the lowest (2.12 ± 0.02 mg/kg) was reported in *Carissa edulis* from the Nyamira study area. Fractionation results indicated that the proportion of iron potentially released (bio-accessible) from all the medicinal plants was quite small. *Clerodendrum myricoides* had the highest mean iron release and solubility (16.4 mg/kg), while *Warburgia ugandensis* had the lowest (5.94 mg/kg) among the medicinal plants. More iron and copper were extracted enzymatically as compared to the aqueous extraction. The amount of iron extracted in the intestinal mimicked phase was more than that extracted in the gastric phase, while the reverse was observed for copper. The levels of bio-accessible iron and copper from the medicinal plants, especially those released in enzymatic extraction are quite high and the plants can be used in the management of anaemia.

Keywords: Medicinal plants, Anaemia, Bio-accessibility, Fractionation

Introduction

The prevalence of anaemia is higher in developing countries than that in developed countries because of poverty, lack of hygiene and the high cost of the disease management [1]. Other factors such as malaria, haemoglobinopathies, nutritional deficiencies, heavy loss of blood and high prevalence of parasitic gastrointestinal infections in Third World countries lead to worse outcomes. Anaemia is a nutritional iron deficiency condition. A third of the world population suffers from iron deficiency,

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making it the most common nutritional deficiency [2], and half of them suffer from severe iron deficiency anaemia [3].

The individuals affected by anaemia in the Third World are on the rise due to depletion of iron levels in the diets, and supplementation programs have not succeeded due to the high costs of iron supplements and other health complicating factors [4]. This has led to the increase in anaemia cases among the residents, especially infants. The drugs for anaemia treatment are not affordable for most residents of countries such as Kenya, and therefore cheap available alternative medicines with the right amount of bio-accessible iron need to be found. The bioavailable and bio-accessible concentrations of iron and copper in the medicinal plants depend on the soil type, rainfall, industrial activity and agricultural activity of the area where the plant grows [5].

Globally, 273 million children and 529 million women were estimated by WHO in 2015 to be affected by anemia [2,3,6]. The prevalence of maternal anaemia is still high in low- and middle-income countries [4,7], with countries in sub-Saharan Africa and Southeast Asia being the most affected [8]. In Kenya, the prevalence of maternal anaemia is also high, and maternal anaemia has not been managed well due to limited resources, leading to morbidity and mortality [4,9,10]. To address this burden, national policy guidelines and interventions on combined iron and folic acid supplementation for pregnant women to improve both neonatal and maternal outcomes have been initiated, though it is not accessible to all the population [4]. Due to diminishing resources, understanding anemia management using the locally available resources will assist local policymakers in utilizing information on the locally available medicinal plants and conducting nutrition-based interventions adapted for individuals in high-risk areas.

Previous studies have reported that some medicinal plants have high total and water-extractable levels of iron and copper. Wesolowk & Koniecynski reported the mean extractable iron (II) levels in Polish medicinal plants ranging from 0.48 ± 0.04 to 16.53 ± 1.15 mg/kg [11], Adeolu Adedapo et al. found 986 mg/100 g in *Bidens pilosa* and 255 mg/100 g in *Chenopodium album* from South Africa [12], and Alikwe found 789 ± 0.1 mg/kg in *Bidens pilosa* from Nigeria [13].

The methods that have been used in the determination of iron and copper in the medicinal plants include Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Flame Atomic Absorption Spectrometry (FAAS), Inductively Coupled Plasma Optical Emission Mass Spectrometry (ICP-OEMS), Sequential Extraction Procedure (SEP) and Enzymatic Extraction Procedure (EEP) [14,15].

The amount of the bioavailable and bio-accessible content of iron and other mineral nutrients obtained from food substance to the human body depends on various factors which enhance or inhibit iron absorption [16,17]. The release of iron from iron-rich diets is approximately 14%-18%, while that from vegetarian diets is 5%-12% [16,17,19]. Large amounts of iron would be lost during menstruation in women due to blood loss. Murray-Kolbe and Beard have reported that heme iron has higher bioavailability than non-heme iron dietary components [20]. When phytates are present in the diet, they inhibit iron absorption [19]. The iron levels in plants differ from one plant species to another, and hence the amount of iron bio-available and bio-accessible to the body from a medicinal plant does not necessarily depend on the total iron content. It is important to determine bio-accessible iron and copper levels to avail more accurate information on the therapeutic activity of the flora.

Divya Sree and Rajasekaran (2021) reviewed 70 medicinal plants used to treat anaemia in developing countries and reported that Sorghum bicolor stem bark, Brillantasia nitens leaves, Tectona grandis, and Allium ascalonicum medicinal plants have the ability to treat anaemia [21]. Peter et al. in 2014 reviewed 28 plant species and revealed that Hibiscus sabdariffa, Lawsonia inermis, *Aloe sp, Uvaria acuminata, Parinari curatellifolia, Ozoroa reticulata, Manihot esculenta, Canthium sp* and *Afzelia quanzensis* plant species from Tanzania are of great importance in anaemia treatment [22]. Kigen et al. (2017) reviewed 99 medicinal plants used by the Markwet community in Kenya and revealed that *B. alba*, *N. officinale*, or *Persea americana* has a potential for anaemia treatment [23]. Treatment of anaemia requires the intake of iron and copper-rich diet, iron, copper and vitamin supplements. Therefore, the total and bio-accessible levels of iron and copper in the traditionally used medicinal plants must be determined to ascertain their curative activity.

Medicinal plants have different habits, which can be used for their identification. *Croton macrostachyus* is a deciduous tree growing to 3-25 m; the bark is grey or grey brown, finely reticulate. The characteristics are as follows: leaves turning orange before falling, ovate base cordate, apex acuminate, margin crenulate-serrulate or subentire, 5-19 by 3.5-15 cm, stellate-hairy but more densely so beneath. Flowers are yellowish, 3.5 mm long, in 15-32 cm long racemes; the flowers in cluster, either all male or all female or mixed, dioecious or monoecious. Fruit is grey, trilobed, 8-9 by 8-10 mm stellate-pubescent. It often flowers when leafless. Leaflets are broadly ovate, round or wider than the length, base rounded to cordate, apex rounded or emarginate, 3-20 by 3-21 cm, usually tomentose or velvet at least beneath, prickles present on the petiole and sometimes on the midribs of the leaflets. Flowers are bright red (pale pink) in dense erect racemes 2-20 cm long; corolla 2.8-5 cm long. Fruit is woody, straight or curved, 4-16 by 1.2-2.4 cm, much constricted between the seeds. Seeds are scarlet and black; leaves pinnately 3 foliolate usually fleshy and glandular stipels; inflorescence mostly pyramidal; and keel and wings usually much smaller [24-26].

Clerodendrum myricoides is a shrub 1-3.5 m tall. The characteristics are: leaves opposite or in threes/fours, ovate or slightly obovate, base cuneate or attenuate, apex acute, margin toothed or rarely entire, to 11 by 5 cm, pubescent to almost glabrous beneath. Flowers blue or purple, occasionally greenish with one lobe blue, or blue with two lobes white; in lax panicles; corolla tube 5-10 mm, lobes 8-17 mm long. Fruit black, 8 mm long [25,27].

Melia azedrach is a deciduous tree around 14-16 meters tall with smooth, greenish-brown bark at a young age, and with age, the bark turns grey and fissured [25,28,29].

Toddalia asiatica is a climbing shrub or liana 2-15 m, and in lianas the lower stem is beset with spines on corky pyramids; branches and often underside of the leaves with hooked prickles to 5 mm. Leaflets elliptic or slightly obovate, base cuneate, apex obtusely acuminate, 3-8 by 1-3 cm, glabrous; margins sometimes crenulate. Flowers are greenish-yellow, in axillary and terminal panicles; petals 2-3 mm long. Fruit is orange, round, 7-10 mm [25,30]. *Aloe vera* has stems 1-2 m, in thickets up to 4 m high. Leaves are 30 by 3.5 cm, yellowish-green, unspotted; teeth 2-5 mm long, 8-10 mm apart. Flowers are red, in 1-4 branched panicles; flowers 30-35 mm long. Fruit is 22 by 9 mm [25,31].

Carissa edulis is a shrub occasionally scrambling, 1-14 m; its bark is grey. Spines are simple, rarely forked, 0.5-5 cm long. Leaves are ovate, elliptic or almost round, base rounded or cuneate, apex obtuse or acute, 1.5-7 by 1.5-4 cm, glabrous or pubescent. Flowers are white inside, pink to red outside, in dense cymes; corolla tube 13-20 mm, lobes 4-9 mm long. Fruit is red to black, round or ellipsoid, 6-14 mm across, sometimes with a sharp apex [25].

Warburgia ugandensis tree is 4.5-30 m tall, evergreen; crown rounded; bark smooth or scaly, pale grey or brown. Leaves are very glossy dark green above, elliptic, often slightly falcate, base cuneate, apex acute or obtuse, 5-15 by 1.4-5 cm, midrib often somewhat eccentric. Flowers are yellow-green, 5-7 mm long. Fruit is ellipsoidal when young, purplish and globose when ripe, 3-5 cm across [25,32].

The purpose of the present study is to assess the bio-accessibility of iron and copper from seven Kenyan anti-anaemia medicinal plants and to evaluate the possibility of iron and copper with high levels in the management of anaemia in Third World countries and worldwide.

Experimental procedures

Recruitment of herbalists and sampling of plant materials

The recruitment details of herbalists practicing in the study areas were described in our previous work [15,33]. Ten herbalists from each study area were recruited from the three study areas and requested to supply 500 grams of dry weight of each plant species. The plant samples were collected between March 2022 and April 2022. The plant species were botanically identified, washed with deionized water to remove soil and other dust material, placed in polythene paper, sealed, air-dried under the shade, and ground using a pestle and wooden mortar to avoid contamination.

This step must be very careful to ensure that there was no contamination during the collection and storage of the herbal plants. Each sample was placed in the transparent polythene bag and stored in a cool, dry area until analysis. The plants used were *Aloe vera (Aloe barbadensis Miller), Carissa edulis, Croton macrostachyus, Clerodendrum myricoides, Melia azedrach, Toddalia asiatica,* and *Warburgia ugandensis.*

Reagents, chemicals and apparatus

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 sequential extraction were acetic acid, hydroxylamine hydrochloride, hydrogen peroxide, ammonium acetate, nitric and hydrochloric acids, while those for enzymatic digestion were pepsin, sodium malate, sodium citrate, lactic acid, acetic acid, bile salts and pancreatin.

The determination of the total amount of copper and iron in plants

Mogwasi et al. (2020) procedure was adopted in the determination of the total concentrations of copper and iron in the anti-anemia medicinal plants [33]. Each sample was analysed in triplicate (n = 3) to test for homogeneity and analytical reproducibility.

Sequential extraction

The modified Bureau Community of Reference (BCR) was used to determine the concentrations of exchangeable, oxidizable, reducible and residual fractions of copper and iron in the plant species [15].

Sequential ultra-filtration for the determination of elements in medicinal plants

Mogwasi et al. (2019) [33] and Nischwitz et al. (2017) [14] procedures were used to classify iron and copper in the medicinal plant extracts into < 3 kDa, 3 kDa-10 kDa, 10 kDa-0.45 µm and 0.45 µm-5 µm mass fractional species. The water extracts and filtrates obtained from sequential filtration were analysed by external calibration using Rh as the internal standard. NIST 1640a natural water reference material and 1547 peach plant reference material were analysed for quality control.

Enzymatic determination of elements in medicinal plants

The physiologically-based extraction procedure of Mogwasi et al. [33] was used to determine the amount of copper and iron extracted enzymatically. 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 of lactic acid and 500 µL of 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. The solution was centrifuged at 3000 rpm for 10 min and 5 mL of aliquot was removed and filtered through 0.45 µm micro filter and replaced with the original gastric acid solution to retain the original solid-solution ratio.

In order to stimulate conditions of the small intestine digestion, 52.5 mg of 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 at 37 °C for 2 h when a second 5 mL of aliquot was removed and filtered. The final sample was used to confirm whether small intestinal equilibrium has 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 samples were diluted (1:10 w/w). Gastric, intestinal and water medicinal plant extracts were performed in every batch, and all the samples were extracted and analyzed in triplicate. The bio-accessible metal contents of plant extracts (gastric and intestinal fractions) were determined by FAAS.

Data analysis

Binomial t-test was used to determine whether the plant species and the study area from which the plant was collected significantly contribute to the levels of copper and iron in the plants. One-way was used to determine whether the differences between the means of copper and iron within the plants of a given study area and the differences among the three study areas are statistically significant. IBM Statistics 22 package was used in the analysis. An α value of 0.05 was adopted as the critical level for all statistical testing giving a 95% confidence level.

Results and discussion

Total levels of iron and copper in anti-anemia plants

The influence of sampling location and plant species on the bio-accessible concentrations of iron and copper in seven anti-anaemia medicinal plants collected from different study sites in Kenya was investigated. Thirty-five medicinal plants from three Kenyan study sites were screened out for total iron and copper levels (elements known to manage anaemia), and seven plant species with sufficient amounts of the elements were selected for the study. FAAS analysis was used to determine the total elemental levels, and the fractional species of the elements were determined by Sequential Extraction Procedure (SEP) and ultra-filtration technology, while Enzymatic Extraction Procedure (EEP) was applied to determine the bio-accessibility of the elements. The total concentrations of iron and copper in the anti-anaemia medicinal plants from the three sub-counties in Kenya were determined by FAAS. The results are presented in Table 1.

Table 1. Total concentrations of iron and copper in the anti-anaemia medicinal plants

Anti-anaemia medicinal plant	Total iron concentration (mg/kg)			Total copper concentration (mg/kg)		
	Nyamira (N)	Borabu (B)	Ekerenyo (E)	Nyamira (N)	Borabu (B)	Ekerenyo (E)
Warburgia ugandensis	175.76 ± 5.03	464.00 ± 2.37	453 ± 2.3	3.73 ± 0.25	4.72 ± 0.21	4.7 ± 0.36
Toddalia asiatica	978.73 ± 8.3	985.76 ± 6.20	983 ± 3.34	3.21 ± 0.03	5.53 ± 0.2	5.2 ± 0.53
Croton macrostachyus	1582.39 ± 5.05	1184.37 ± 0.38	1207 ± 3.33	4.82 ± 0.23	7.76 ± 1.5	8.1 ± 1.23
Melia azedarach	1756.36 ± 8.95	1004.30 ± 7.8	923 ± 4.33	3.88 ± 0.15	8.94 ± 1.5	9.2 ± 1.24
Clerodendrum myricoides	985.40 ± 5.06	1197.02 ± 14	1242 ± 5.83	8.61 ± 0.16	6.25 ± 1.5	5.5 ± 0.33
Carissa edulis	2143.23 ± 9.3	2635.48 ± 17.33	2529 ± 6.76	2.12 ± 0.02	7.13 ± 1.4	8.1 ± 0.48
Aloe vera	1313.59 ± 3.72	1243.22 ± 9.41	1303 ± 4.52	3.88 ± 0.91	3.2 ± 0.63	2.99 ± 0.318

Table 2. The fractions of iron and copper in anti-anaemia medicinal plants

Anti-anaemia medicinal plant			Step 1	Step 2	Step 3	Step 4	Total digestion (mg/kg)	Recovery %	EBF %	PBF %
Melia azedarach	N	Fe	28.24	1,528.31	18.85	5986.91	8004.6	94.1	0.4	19.3
		Cu	0.58	2.17	0.73	7.85	12.92	87.8	4.5	22.5
	B	Fe	9.95	207.44	29.63	757.28	1086.90	92.4	0.9	21.8
		Cu	0.71	2.68	1.61	7.73	14.23	89.4	5.0	30.2
	E	Fe	8.82	172.05	25.03	728.10	973.93	95.9	0.9	20.2
		Cu	0.69	2.63	1.96	7.1	13.90	92.8	5.0	33.0
Warburgia ungandensis	N	Fe	1.35	16.48	5.66	139.27	203.43	86.4	0.7	10.9
		Cu	0.17	0.60	0.23	2.73	4.09	91.2	4.2	20.3
	B	Fe	9.12	108.41	9.77	246.70	530.89	87.4	1.7	22.3
		Cu	0.11	1.38	0.53	2.69	5.46	86.4	2.0	35.0
	E	Fe	7.29	62.11	35.55	298.05	591.99	79.9	1.2	16.5
		Cu	0.34	1.22	0.52	2.92	6.09	82.1	5.6	28.6
Croton macrostachyus	N	Fe	5.49	56.34	27.71	842.86	2746.80	75.9	0.2	3.1
		Cu	0.21	1.36	0.22	3.10	7.67	79.3	2.7	20.6
	B	Fe	6.95	341.73	3.06	832.63	1375.57	86.1	0.5	25.1
		Cu	0.23	1.80	0.49	5.08	8.31	91.5	2.8	27.6
	E	Fe	8.07	161.52	6.82	1041.59	1553.57	78.4	0.5	10.8
		Cu	0.46	1.81	0.78	5.35	9.51	88.3	4.8	27.2
Clerodendrum myricoides	N	Fe	9.92	294.54	4.31	676.63	1104.71	89.2	0.9	27.1
		Cu	0.09	1.66	0.25	6.62	9.15	94.1	1.0	20.9
	B	Fe	31.24	121.02	12.14	1032.62	1421.64	84.2	2.2	9.4
		Cu	0.35	1.30	0.75	3.85	7.23	86.4	4.8	28.4
	E	Fe	8.04	347.97	18.68	878.31	1430.36	87.6	0.6	25.6
		Cu	0.29	1.17	0.48	3.86	6.15	94.3	4.7	26.8
Aloe vera	N	Fe	8.26	490.71	116.23	698.39	1379.82	95.2	0.6	44.0
		Cu	0.37	1.30	0.59	2.67	5.67	86.7	6.5	33.2
	B	Fe	8.40	277.76	120.88	836.18	1280.35	97.1	0.7	31.1
		Cu	1.11	3.39	1.98	7.44	16.11	86.4	6.9	33.3
	E	Fe	7.83	396.71	19.11	889.35	1402.78	93.6	0.6	29.6
		Cu	0.67	2.29	1.15	10.19	15.20	94.1	4.4	22.6
Toddalia asiatica	N	Fe	13.34	543.76	235.26	1,415.84	2503.64	88.2	0.5	31.2
		Cu	1.03	1.54	1.23	9.24	14.85	87.8	6.9	18.7
	B	Fe	8.70	89.11	85.90	802.05	1074.98	91.7	0.8	16.3
		Cu	0.24	1.78	1.85	7.86	13.19	88.9	1.8	27.5
	E	Fe	17.23	293.19	350.64	1373.94	2556.53	79.6	0.7	25.2
		Cu	0.75	0.23	1.35	6.22	10.98	77.9	6.8	14.4
Carissa edulis	N	Fe	5.83	83.11	4.94	537.49	669.54	94.3	0.9	13.2
		Cu	0.30	1.13	0.63	4.74	6.8	82.3	4.4	25.9
	B	Fe	8.58	75.21	14.27	323.50	452.81	93.1	1.9	19.8
		Cu	0.15	1.80	0.75	4.43	8.20	86.9	1.8	31.1
	E	Fe	9.15	90.80	12.69	355.01	449.95	92.9	2.0	23.0
		Cu	0.23	1.13	0.89	5.95	9.94	82.5	2.3	20.3

Iron

Iron levels in the medicinal plants from Borabu ranged from 464.00 ± 2.37 mg/kg to 2635.48 ± 17.33 mg/kg. The four medicinal plants from the area with high iron levels were *C. edulis* (2635 mg/kg), *A. vera* (1243 mg/kg), *C. myricoides* (1197 mg/kg) and *C. macrostachyus* (1184mg/kg). The mean iron levels in medicinal plants from Ekerenyo ranged from 411 ± 2.33 to 2529 ± 6.76 mg/kg, among which *C. edulis* (2529 mg/kg), *A. vera* (1303 mg/kg), *C. myricoides* (1242 mg/kg), and *C. macrostachyus* (1207 mg/kg) were four medicinal plants with high iron levels. The mean iron levels in Nyamira medicinal plants ranged from 175.76 ± 5.03 to 2143.23 ± 9.3 mg/kg, with *C. edulis* (2143 mg/kg), *M. azedarach* (1756 mg/kg), *C. macrostachyus* (1582 mg/kg) and *A. vera* (1313 mg/kg) as the medicinal plants with high iron levels. The highest mean levels of iron were recorded in *C. edulis* from the three sub-counties, while the lowest were in *W. ugandensis*. Iron levels in the medicinal plants were statistically significant compared with those of copper in Nyamira and Ekerenyo, while the levels of iron in the medicinal plants in the sub-counties were not statistically different from each other ($P < 0.05$) [Table 3&4].

Table 3. Correlation coefficient (r) and t-test for the total levels of Fe with Cu in the medicinal plants

	Fe		Cu		Fe & Cu	
	r-test	t-test	r-test	t-test	r-test	t-test
N/B	0.788	-0.433	0.028	0.332		
N/E	0.773	0.058	0.149	-1.010		
B/E	0.996	0.058	0.976	-0.386		
N					0.284	1.361
B					0.242	1.495
E					0.310	1.421

The soils of Nyamira County are generally ferruginous with high cation exchange capacity, enabling the plants to absorb high levels of iron from the soil, as depicted from the obtained results. The levels of iron obtained in our study are comparable with those of Adedapo et al. of 986 mg/100 g in *Biden pilosa* and 255 mg/100 g in *Chenopodium album* in South Africa [13] and Alikwe et al. of 789 ± 0.1 mg/kg in *B. plosa* from Nigeria [18]. The four anti-anaemia plants from study sites rich in iron were *C. edulis*, *M. azedarach, A. vera* and *T. asciatica*. The iron in the medicinal plants enhances the synthesis of the erythrocytes. Most medicinal plants used by the herbalists to manage anaemia were found to have high levels of iron.

Copper

The mean copper levels in Borabu medicinal plants ranged from 3.2 ± 0.63 to 8.94 ± 1.5 mg/kg with *M. azedarach* (8.94 mg/kg), *C. macrostachyus* (7.76 mg/kg), *C. edulis* (7.13 mg/kg), and *C. myricoides* (6.25 mg/kg) having high copper levels. The copper levels in Ekerenyo medicinal plants ranged from 2.99 ± 0.318 to 9.2 ± 1.24 mg/kg. The four medicinal plants from Ekerenyo with high copper levels were *M. azedarach* (9.2 mg/kg), *C. macrostachyus* (8.1 mg/kg), *C. edulis* (8.1 mg/kg) and *C. myricoides* (5.5 mg/kg), while the Nyamira medicinal plants had higher copper contents ranging from 2.12 ± 0.02 to 8.61 ± 0.16 mg/kg including *C. myricoides* (8.61 mg/kg), *C. macrostachyus* (4.82 mg/kg), *A. vera* (3.88 mg/kg) and *M. azedarach* (3.88 mg/kg). The highest mean content of copper was recorded in *M. azedarach* from Borabu and Ekerenyo and in *C. myricoides* from Nyamira. The highest mean level of copper of 9.2 ± 1.24 mg/kg was recorded in *M. azedarach* from Ekerenyo, while the lowest level of 2.12 ± 0.02 mg/kg was recorded in *C. edulis* from Nyamira. The levels of copper in the medicinal plants were statistically different from those of iron in the study areas ($P < 0.05$), while the copper levels in the medicinal plants in the sub-counties were not statistically significant from each other ($P < 0.05$) (Table 3&4). The levels of copper in the medicinal plants in the present study were similar to those reported by Anna et al. of 9.40-16.70 mg/100 g among the Brazilian teas [34] and Adedapo et al. of 24 mg/100 g in the South African medicinal plants [13] as they have similar climatic conditions.

Copper has been shown to be involved in homeostasis, bringing about immunity and iron metabolism in the human body [35-37]. Iakovidis et al. have reported the use of copper complexes as anti-inflammatory drugs has low side effects [35]. Masako and Yoshiyuki have reported the antimicrobial, antiviral, anti-inflammatory, antitumor agents, enzyme inhibitors, or chemical nuclease activities of copper complexes [38]. This means that those plants which have been shown to have high copper levels need to be subjected to bioassay tests for their bioactivities (antimicrobial, antiviral, anti-inflammatory, antitumor agents, enzyme inhibitors, or chemical nuclease activities).

The difference in subject sites is highly positively correlated with the total amount of iron in the medicinal plants, while the content of copper is higher only in Borabu and Ekerenyo (Table 3). This means consumption of the medicinal plants from the areas supply the consumer with almost similar amounts of the elements. The correlation of copper in medicinal plants from Ekerenyo, Nyamira and Borabu was quite low. This could be related to the microclimatic conditions of the sample collection sites.

Table 4. Anova for Fe vs Cu medicinal plants in Nyamira county

		Sum of Squares	Df	Mean Square	F	Sig.
Ekerenyo	Between Groups	19046014.105	5	1190375.882	0.162	0.990
	Within Groups	14718698.000	2	7359349.000		
	Total	33764712.105	7			
Borabu	Between Groups	5.323	5	0.409	1.597	0.317
	Within Groups	1.282	2	0.256		
	Total	6.604	7			
Nyamira	Between Groups	35544222.947	6	2090836.644	6.219	0.307
	Within Groups	336200.000	1	336200.000		
	Total	35880422.947	7			

Sequential extraction of iron and copper

The various forms of iron and copper were sequentially extracted from the medicinal plants from Nyamira sub-counties and determined by FAAS. The results are presented in Table 2. The residual fraction was more than reducible fraction which was more than the oxidizable fraction, while the exchangeable fraction of iron was the least. The information on the forms (species) of the metal in the medicinal plant is essential for predicting the therapeutic activity of the plant. The level of solubility and the release metal from the medicinal plant were measured. The bioavailable fractions of iron in these plants were comparable with those reported by Omolo et al. in other Kenyan medicinal plants [39]. However, the Nigerian medicinal plants were reported by Majolagbe et al. to have higher bioavailable fractions [40]. Li & Deng also pointed out that the bioavailable form of the metal obtained depends on the extraction solvent, plant part used and the plant species [41]. Koniecynski et al. [11] further reported that the extracted Fe (II) by water from medicinal plants is a source of the bioavailable form of iron to human beings.

The highest percentage of recovery of iron in the present study was 97.1% in *Aloe vera* from Borabu, while the lowest was 75.9% in *C. macrostachyus* from Nyamira. The highest easily bioavailable fraction (EBF) of iron (2.2%) was in *C. myricoides* from Borabu and the lowest (0.2%) was in *C. macrostachyus* from Nyamira. The highest potential bioavailable fraction (PBF) of iron of (44.0%) was in *A. vera* from Nyamira and the lowest (3.1%) in *C. macrostachyus* from Nyamira (Table 2). The form in which iron is consumed affects the dietary intake of an individual. The factors that determine the proportion of iron absorbed from the diet (and medicinal plant) are complex. They include the iron status of an individual and the iron content of the meal. The aqueous solubility and release of iron in the present study from the medicinal plants varied from 0.002 to 0.005 percent of the total iron levels in the plants. The mean percentages of solubility and release (exchangeable) of iron from aqueous media for the medicinal plants were: *C. edulis* (1.6), *C. myricoides* (1.23), *W. ugandensis* (1.2), *M. azedarach* (0.73), *T. asiatica* (0.667), *A. vera* (0.633) and *C. macrostachyus* (0.4) (Table 2). *C. myricoides* (16.4 mg/kg), *M. azedarach* (15.67 mg/kg) and *T. Asiatica* (13.09 mg/kg) were the three medicinal plants with high mean amounts of solubility of iron, while those with high released amounts by mass were *C. myricoides* (31.24 mg/kg) from Borabu, *M. azedarach* (28.24 mg/kg) from Nyamira and *T. asciatica* (17.23 mg/kg) from Ekerenyo. The percentage of release and the total mass of the iron level in the medicinal plant must be considered in the selection of the plant to be used in anemia management. The plants which had high total levels of iron were not the ones which had high exchangeable levels. The plants which have high exchangeable levels of iron can be used in the management of anemia. The medicinal plants provide the iron naturally to the body, so there are no adverse effects compared with artificial supplements in the form of tablets [14].

The presence of other nutrients such as vitamin C and organic acids such as citric, lactic or malic acid can increase the absorption of iron, while calcium, zinc, phytates, polyphenols and vegetable protein inhibit the absorption of non-heme iron [17,19,20].The iron absorbed must be lower than the easily bioavailable forms of iron in the medicinal plants as it is influenced by the individual diet.

The trends in decreasing order of various fractions of iron are residual > reducible > oxidizable > exchangeable forms (species) in the medicinal plant species. Substantial amounts of iron are associated with residual fraction in the medicinal plants, indicating that iron is strongly bound in the plant tissues. Given the effectiveness of the various medicinal plants under similar conditions to act as a source of iron and in blood-building, the management of anaemia based on the bioavailability of iron is in the order: *M. azedarach* > *T. asiatica* > *C. macrostachyus* > *C. myricoides* > *A. vera* > *W. ungandensis*.

The highest percentage of recovery of copper (94.3%) was in *C. myricoides* from Ekerenyo, while the lowest (79.3%) was in *C. macrostachyus* from Nyamira. The highest EBF of copper (6.9%) was in *A. vera* from Borabu and *T. asiatica* from Nyamira, while the lowest (1.0%) was in *C. myricoides* from Nyamira. The highest PBF of copper (33.30%) was in *A. vera* from Borabu and the lowest (18.7%) in *T. asiatica* from Nyamira (Table 2). These results are comparable with those reported for Cr, Mn and Zn in our previous work in twelve anti-diabetic medicinal plants from Nyamira County, Kenya [15].

Ultra-filtration of iron and copper in the medicinal plants

The hot water extracts of three medicinal plants (*C. macrostachyus, C. edulis and A. vera*) were subjected to a 4-step sequential filtration procedure, resulting in the following size fractions as described in the experimental section: < 3 kDa, 3 kDa-10 kDa, 10 kDa-0.45 µm and 0.45 µm-5 µm, and the iron and copper species were determined by FAAS. An elemental analysis of the fractions clearly showed that the mass distribution of the two elements across these fractions was strongly dependent on the plant species. The results for the elements are summarized in Figure 1a&1b.

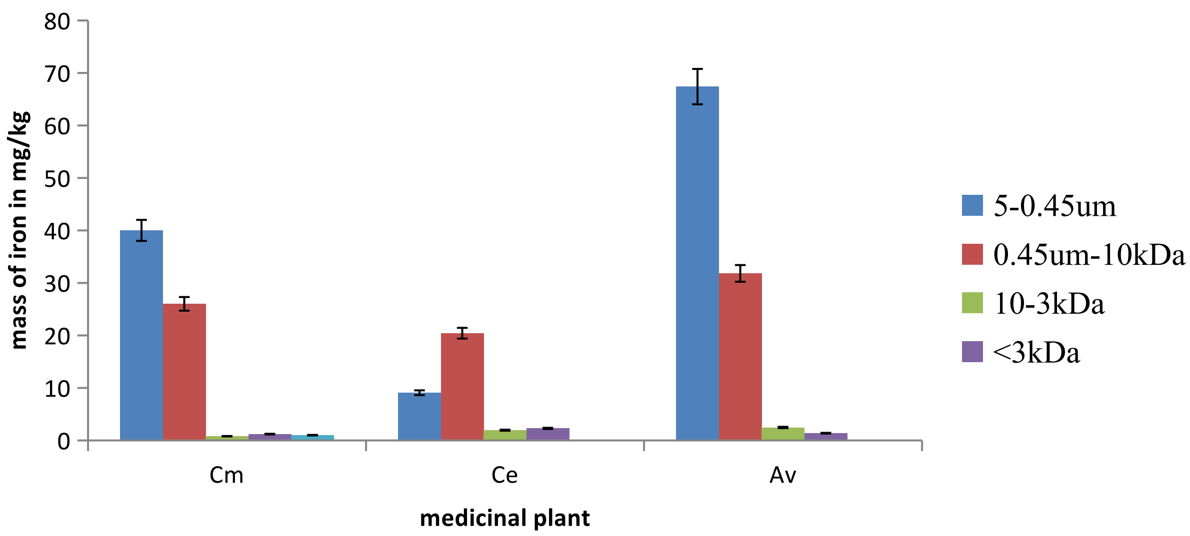

Figure 1a: Fractional species of iron in anti-anaemia medicinal plants from Kenya

The molecular-size of the mass species distributions of iron and copper in the anti-anemia medicinal plants were different in the plants. In *C. edulis*, the 0.45 µm-10 kDa molecular-size iron species were of the highest occurrence, while the < 3 kDa molecular-size species were of the lowest occurrence. In *C. myricoides* and *A. vera*, the > 0.45 µm molecular-size species were of the highest occurrence, while 10 kDa-3 kDa molecular-size mass species were of the lowest occurrence in *C. myricoides*, and the < 3 kDa molecular mass species were of the lowest occurrence in *A. vera*. Iron in most plants occurred in the > 0.45 µm molecular-size mass species (Figure 1a).

In *C. myricoides*, *C. edulis* and *A. vera*, the 0.45 µm-10 kDa molecular-size copper species were of the highest occurrence, while 5-0.45 µm molecular copper species were of the lowest occurrence in *C. myricoides* and absent in *C. edulis* and *A. vera* (Figure 1b).

Physiologically-based extractions of iron and copper from the anti-anemia medicinal plants

The levels of iron and copper in the anti-anemia medicinal plants were enzymatically and aquatically extracted. The gastrointestinal digestion was stimulated, and the amounts of each element were determined by FAAS.

The highest extracted iron in the gastric phase (6.2 g/kg) were in *A. vera* and *M. azedarach*, and the lowest was in *W. ugandesis* (1.8 g/kg). The highest extracted iron in the intestinal phase was in *C. edulis* (13.82 g/kg) and the lowest

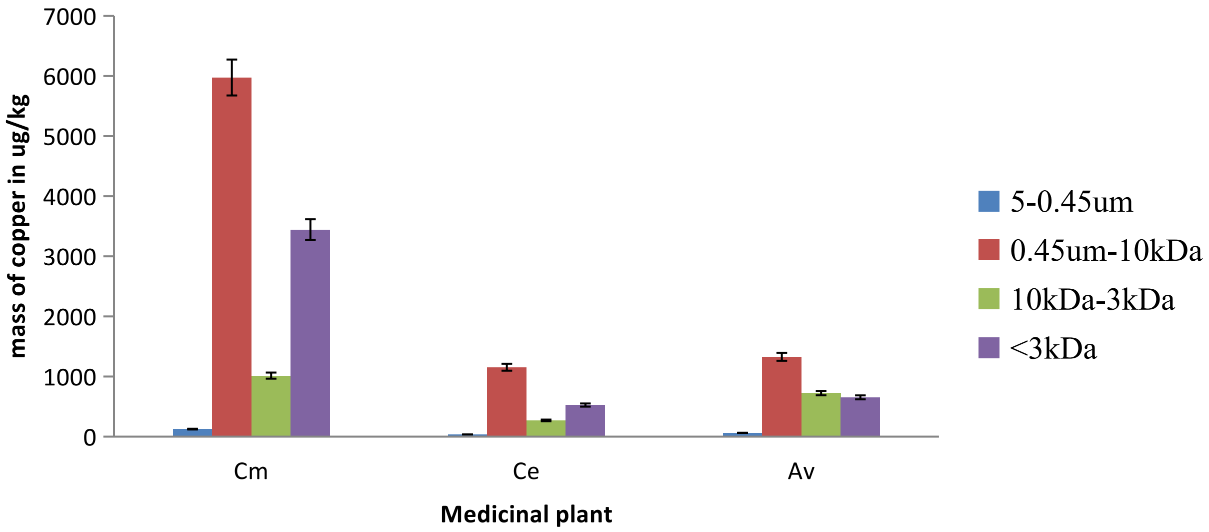

Figure 1b: Fractional species of copper in anti-anaemia medicinal plants from Kenya

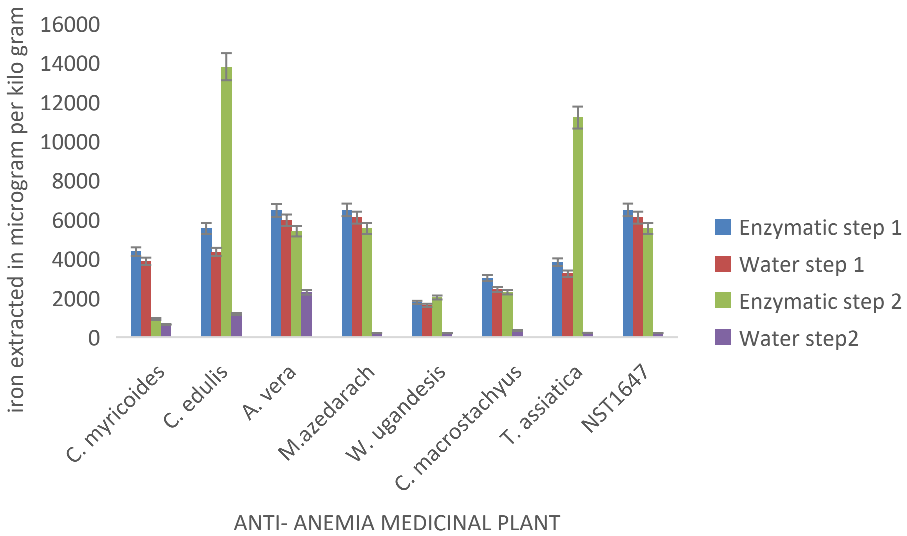

Figure 2a: Comparison of aqueous and enzymatic extraction as the first step to characterize iron species present in plants

was in *W. ugandesis* (1.8 g/kg). The highest total enzymatically extracted iron was from *C. edulis* (19.62 g/kg) and the lowest was in *W. ugandesis* (3.6 g/kg), while the highest total aquatically extracted iron was in *M. azedarach* (8.8 g/kg) and the lowest in *W. ugandesis* (1.85 g/kg) (Figure 2a).

The highest amount of copper extracted in the gastric phase was in *C. myricoides* (1.39 g/kg) and the lowest in *W. ugandesis* (0.6 g/kg), while the highest amount extracted in the intestinal phase was in *T. asiatica* (0.82 g/kg) and the lowest in *A. vera* (0.3 g/kg). The highest total aquatically extracted iron was in *C. myricoides* (2.01 g/kg) and the lowest in *W. ugandesis* (0.61 g/kg), while the highest total aquatically copper extracted was in *C. myricoides* (1.46 g/kg) and the lowest in *C. macrostachyus* (0.5 g/kg) (Figure 2b).

The amounts of iron and copper extracted enzymatically were more than those extracted in water in the plants. This means that the potentially bioavailable fraction of the elements is absorbed by the human body when the medicinal plants are consumed. Though the amount obtained depends on the diet composition of the individual. Our results are similar to those reported by Cadkova et al. [42], which showed that the amount of iron and copper extracted in the gastric phase was more than that extracted in the intestinal phase. The copper complexes with a number of organic ligands that break down into different extents. Similar elements in dissimilar biological matrices bound to compounds to form complexes with different degradability with digestive enzymes. The behavior of iron

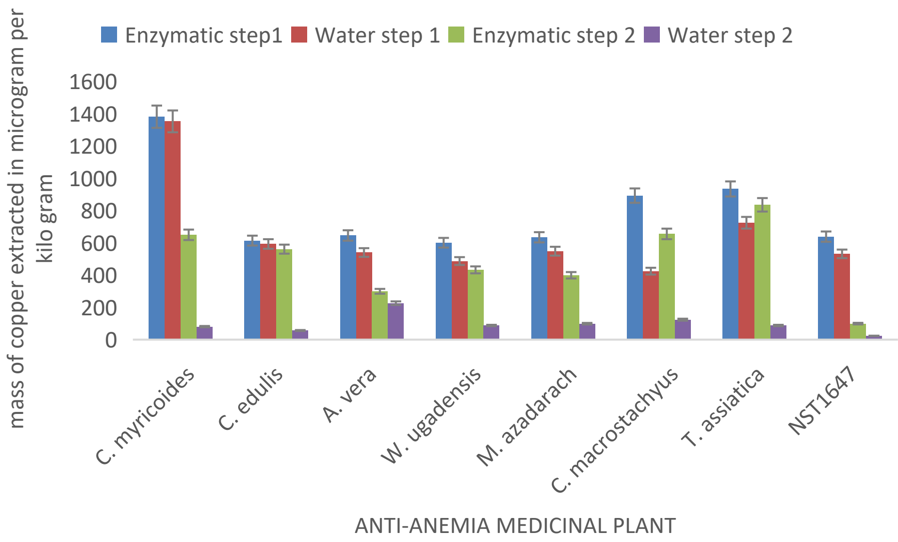

Figure 2b: Comparison of aqueous and enzymatic extraction as the first step to characterize copper species present in plants

differs slightly from that of copper as it has low bio-accessibility under simulated stomach conditions due to the high concentration of phytates and phytosterols, which strongly bind to iron in acidic environment. Iron bio-accessibility 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 medicinal plant facilitates the conversion of iron (II) into iron (III), which is used in the synthesis of erythrocytes, the synthesis of blood, thus managing anemia.

Conclusion

The residual fraction of iron in the medicinal plants was the highest, while the exchangeable species in the medicinal plants were the lowest. Based on our findings, it can be concluded that *M. azedarach*, *T. asiatica*, *C. macrostachyus*, *C. myricoides* and *A. vera* have a high easily bioavailable fraction of iron content and can be used in blood building and hence in the management of anaemia. The speciation pattern based on sequential extraction reveals that a substantial amount of iron in the plants is associated with the residual fraction. The order of effectiveness of the various plants investigated based on the bioavailability as a source of iron and anaemia management is *M. azedarach* > *T. asiatica* > *C. macrostachyus* > *C. myricoides* > *A. vera* > *W. ungandensis*. According to studies, the consumption of the medicinal plants supplies the iron used in the management of anaemia; the iron in the medicinal plants is used in the synthesis of the erythrocytes, which are destroyed by the plasmodium parasites when one is afflicted by malaria, a prevalent condition leading to anaemia in the studied area. The anti-anaemia potential of the plants could be the presence of iron in the plants among other factors. This demonstrates that the herbalists, though ignorant of the chemical composition of the plants, are able to manage anaemia by their choice of medicinal plants.

Conflicts of interest

There is no conflict of interest whatsoever.

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