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Indigenous leafy vegetables of Eastern Africa — A source of extraordinary secondary plant metabolites

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ABSTRACT

Indigenous African leafy vegetables vary enormously in their secondary plant metabolites whereat genus and the species have a great impact. In African nightshade (*Solanum scabrum*), spiderplant (*Cleome gynandra*), amaranth (*Amaranthus cruentus*), cowpea (*Vigna unguiculata*), Ethiopian kale (*Brassica carinata*) and common kale (*Brassica oleracea*) the specific secondary metabolite profile was elucidated and gained detailed data about carotenoids, chlorophylls, glucosinolates and phenolic compounds all having an appropriate contribution to health beneficial properties of indigenous African leafy vegetables. Exemplarily, various quercetin glycosides such as quercetin-3-rutinoside occur in high concentrations in African nightshade, spiderplant, and amaranth between ~1400–3300 μg/g DW. Additionally the extraordinary hydroxycinnamic acid derivatives such as glucaric isomers and isocitric acid isomers are found especially in amaranth (up to ~1250 μg/g DW) and spiderplant (up to 120 μg/g DW). Carotenoids concentrations are high in amaranth (up to101.7 μg/g DW) and spiderplants (up to 64.7 μg/g DW) showing high concentrations of β-carotene, the pro-vitamin A. In contrast to the ubiquitous occurring phenolics and carotenoids, glucosinolates are only present in the Brassicales species Ethiopian kale, common kale and spiderplant characterized by diverse glucosinolate profiles. Generally, the consumption of a variety of these indigenous African leafy vegetables can be recommended to contribute to different benefits such as antioxidant activity, increase pro-vitamin A and anticancerogenic compounds in a healthy diet.

1. Introduction

The African diet comprises of many different ingredients, including leafy vegetables, which contribute to a healthy diet (Baldermann et al., 2016). Apart from playing an important role in the diet, indigenous African leafy vegetables also sponsoring the local economy (Lenne & Ward, 2010), provide environmental services (Frison, 2016b) and are an integral part of African traditional medicine (Padulosi, Thompson, & Rudebjer, 2013). Up to very recently, indigenous African leafy vegetables were regarded in most African communities as food for the poor (Padulosi et al., 2013), despite these advantages. Interestingly, this ideology is rapidly changing. Indigenous leafy vegetables are presently in vogue in some communities in Eastern Africa, and gaining popularity among consumers. In Kenya for example, indigenous African leafy vegetables are sold in large supermarkets in Nairobi, and seed companies are increasingly paying attention towards breeding traditional varieties (Cernansky, 2015). The cultivation area for these vegetables in Kenya increased by 25% between 2011 and 2013 (Cernansky, 2015), also due to increased demand for indigenous African leafy vegetables as people become aware of the benefits of these vegetables. It is therefore common nowadays to find in restaurants, hotels and public canteens, vegetable dishes based on African nightshade (*Solanum scabrum*), spiderplant (*Cleome gynandra*), amaranth (*Amaranthus cruentus*), cowpea (*Vigna unguiculata*), Ethiopian kale (*Brassica carinata*) and common kale (*Brassica oleracea*) cooked solely or in different combinations.

Sub-Saharan Africa with over 10% of the world's population has the highest occurrence of undernutrition in the world (FAO, 2014), with over 70% of rural population who depend mainly on self-produced foods. This situation is typical in Eastern African. Incidences of diet-related diseases like various cancers and cardiovascular diseases are also increasing in these communities (Tullao, 2002). A practical and sustainable option for addressing this multiple burden of malnutrition in such communities is by exploiting the potential of local biodiversity especially with regard to vegetables. The nutritive benefits of a more diversified diet are presently widely recognized (Frison, 2016a). In addition to their richness in minerals and vitamins (Nesamvuni, Steyn, & Potgieter, 2001;

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Yang & Keding, 2009), indigenous African leafy vegetables have been shown to possess anti-oxidative properties and thus have the potential as natural sources for reducing cellular oxidative damage, and suppression of various cancers and cardiovascular diseases (Uusiku, Oelofse, Duodu, Bester, & Faber, 2010). These anti-oxidative properties are due to the presence of a diversity of secondary plant metabolites. However, there is a very limited knowledge on the secondary plant metabolites in indigenous African leafy vegetables and how these contribute to human nutrition and health. Due to the plant species selected flavonoids and hydroxycinnamic acids, carotenoids and chlorophylls are expected in all selected species, whereas and glucosinolates are limited to the Brassicales species.

Flavonoids and hydroxycinnamic acid derivatives are ubiquitous in plants and belong to the group of polyphenols (Mierziak, Kostyn, & Kulma, 2014). The main dietary flavonoids are quercetin and kaempferol present as glycosides in the plant while the main hydroxycinnamic acid is chlorogenic acid. They are of interest due to their antioxidant activity (Zietz et al., 2010), anti-inflammatory and anticancerogenic effects on humans (Chen & Chen, 2013; Pan, Lai, & Ho, 2010). Till now, a detailed hydroxycinnamic acid and flavonoid glycoside identification and quantification has not been done in indigenous African leafy vegetables.

In general, all carotenoids have anti-oxidative properties and are associated with a less likely risk of the development of cancer. High uptake of carotenoids is linked to a reduction of cardiovascular diseases (Kopsell & Kopsell, 2006). Some carotenes have pro-vitamin A activity and lutein and zeaxanthin are important eye pigments. High uptake of the latter ones likely reduces the risk of age related eye diseases possibly by protection against oxidative damage. Vitamin A deficiency is the leading cause of preventable blindness in children and increases the risk of disease and death from severe diseases in more than half of all countries, especially in South-East Asia and Africa including Kenya (WHO, 2016). Recently, the WHO estimates that 250 million preschool children are vitamin A deficient and 250,000 to 500,000 vitamin A-deficient children become blind every year (WHO, 2016). Besides supplementation the frequent consumption of pro vitamin A rich vegetables could contribute to improve the vitamin status in rural families. Moreover, a rare number of publications indicate that high uptake of chlorophylls, which co-occur with carotenoids in green leafy vegetables, are associated with reduced risk of certain cancers (Gomaa, Ali, El-Tayeb, & Abdel-kader, 2012). In this context, the indigenous African leafy vegetables could have an important contribution as sources of pro vitamin A, lutein and zeaxanthin as well as chlorophylls.

Moreover, several indigenous African leafy vegetables, such as various cabbage types as well as spiderplant, belong to the order Brassicales which is characterized by a specific group of secondary plant metabolites – the glucosinolates (Verkerk et al., 2009). Glucosinolates themselves are not bio-active, but the evidence was found that some of their corresponding break down product – especially the isothiocyanates – demonstrating anticancerogenic (Lippmann et al., 2014), anti-inflammatory (e.g. Herz et al., 2016) or antidiabetogenic (Guzmán-Pérez et al., 2016; Waterman et al., 2015) properties. Therefore, species containing glucosinolates are of interest for their potential health-promoting effects.

In the context of indigenous African leafy vegetables, there is very little research-based evidence on the profile and richness in these secondary plant metabolites. The present study aims to identify and quantify the plant secondary metabolites in six selected species from Eastern Africa, comprising of five indigenous, and one exotic species (common kale), and focusing on the highly interesting flavonoids and hydroxycinnamic acids, carotenoids, chlorophylls and glucosinolates. Five of the selected vegetables are commonly consumed locally namely African nightshade (*S. scabrum*), spiderplant (*C. gynandra*), amaranth (*A. cruentus*), cowpea (*V. unguiculata*) and common kale (*B oleracea*), while the sixth (Ethiopian kale (*B. carinata*)) is being promoted in urban horticulture across the African continent (Ambrose-Oji, 2009).

2. Material and methods

2.1. Plant material

Seeds of the five indigenous African leafy vegetables were supplied by the World Vegetable Center (AVRDC) Arusha, Tanzania, while common kale seeds were bought from a commercial producer (Simlaw Seeds, Nairobi, Kenya). The seeds were sown directly on an experimental field plot at Jomo Kenyatta University of Agriculture and Technology demonstration farm, Juja, Kenya (lat. 1°18′ N; long. 37°12′ N) with a spacing of 20 × 30 cm. The soil is described as a clay soil with a pH of about 5.2. Mean annual rainfall in Juja is about 1500 mm, and temperature of about 20 °C averaged the years 2000–2012 (Worldweatheronline, 2016). Before planting, the plot was supplied with 2 kg/m^2 well-decomposed manure, and 5 g diammonium phosphate per planting hole. Two weeks after planting, the seedlings were additionally fertilized with 6 g calcium ammonium nitrate per plant as recommended based on soil analyses. During the course of the experiment, the plants were irrigated manually twice a day with tap water. Plants were grown for 6 weeks between October and November 2014. At harvest (8–12 leaf stage), fully developed leaves were collected in duplicate per crop species shown as sample 1 and 2 in the graphs and tables. Each replicate consisted of about 300 g of fresh leaf material from about 10 plants selected randomly on the field. The leaves were then freeze-dried and transported to IGZ Großbeeren, Germany, for analyses.

2.2. Flavonoid and hydroxycinnmaic acid analysis

Flavonoids were analyzed according to Schmidt et al. (2010) with slight modification. Lyophilized, ground plant material (0.02 g) was extracted with 600 μL of 60% aqueous methanol on a magnetic stirrer plate for 40 min at 20 °C. The extract was centrifuged at 4500 rpm for 10 min at the same temperature, and the supernatant was collected in a reaction tube. This process was repeated twice with 300 μL of 60% aqueous methanol for 20 min and 10 min, respectively; the three corresponding supernatants were combined. The extract was subsequently evaporated until it was dry and was then suspended in 200 μL of 10% aqueous methanol. The extract was centrifuged at 3000 rpm for 5 min at 20 °C through a Corning® Costar® Spin-X® plastic centrifuge tube filter (Sigma Aldrich Chemical Co., St. Louis, MO, USA) for the HPLC analysis. Each extraction was carried out in duplicate.

Flavonoid composition (including hydroxycinnamic acid derivatives and glycosides of flavonoids) and concentrations were determined from the filtrate using a series 1100 HPLC (Agilent Technologies, Waldbronn, Germany) equipped with a degaser, binary pump, autosampler, column oven, and photodiode array detector. An Ascentis® Express F5 column (150 mm × 4.6 mm, 5 μm, Supelco) was used to separate the compounds at a temperature of 25 °C. Eluent A was 0.5% acetic acid, and eluent B was 100% acetonitrile. The gradient used for eluent B was 5–12% (0–3 min), 12–25% (3–46 min), 25–90% (46–49.5 min), 90% isocratic (49.5–52 min), 90–5% (52–52.7 min), and 5% isocratic (52.7–59 min). The determination was conducted at a flow rate of 0.85 ml min-1 and a wavelength of 280 nm, 320 nm, 330 nm, 370 nm and 520 nm. The hydroxycinnamic acid derivatives and glycosides of flavonoids were tentative identified as deprotonated molecular ions and characteristic mass fragment ions according to Schmidt et al. (2010) and Neugart, Rohn, and Schreiner (2015)) by HPLC-DAD-ESI-MS^n using an Bruker amazon SL ion trap mass spectrometer in negative ionization mode. For the identification of the peaks the data were compared to the literature of the investigated species and their relatives. In the mass spectrometer nitrogen was used as the dry gas (10 L min-1, 325 °C) and the nebulizer gas (40 psi) with a capillary voltage of −3500 V. Helium was used as the collision gas in the ion trap. The mass optimization for the ion optics of the mass spectrometer for quercetin was performed at *m/z* 301 or arbitrarily at *m/z* 1000. The MSn experiments were

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performed in auto up to MS3 in a scan from *m/z* 200–2000. Standards (chlorogenic acid, quercertin 3-glucoside, kaempferol 3-glucoside and; isorhamnetin-3-glucoside Roth, Karlsruhe, Germany) were used for external calibration curves in a semi-quantitative approach. Results are presented as $\mu g\ g^{-1}$ dry weight.

2.3. Carotenoid analysis

Carotenoids were extracted from 5 mg of lyophilized ground leaves with MeOH:THF solution. (1:1 v:v, 500 μL, 3 times). until colorlessby shaking at 1000 rpm for 5 min and followed by centrifugation at

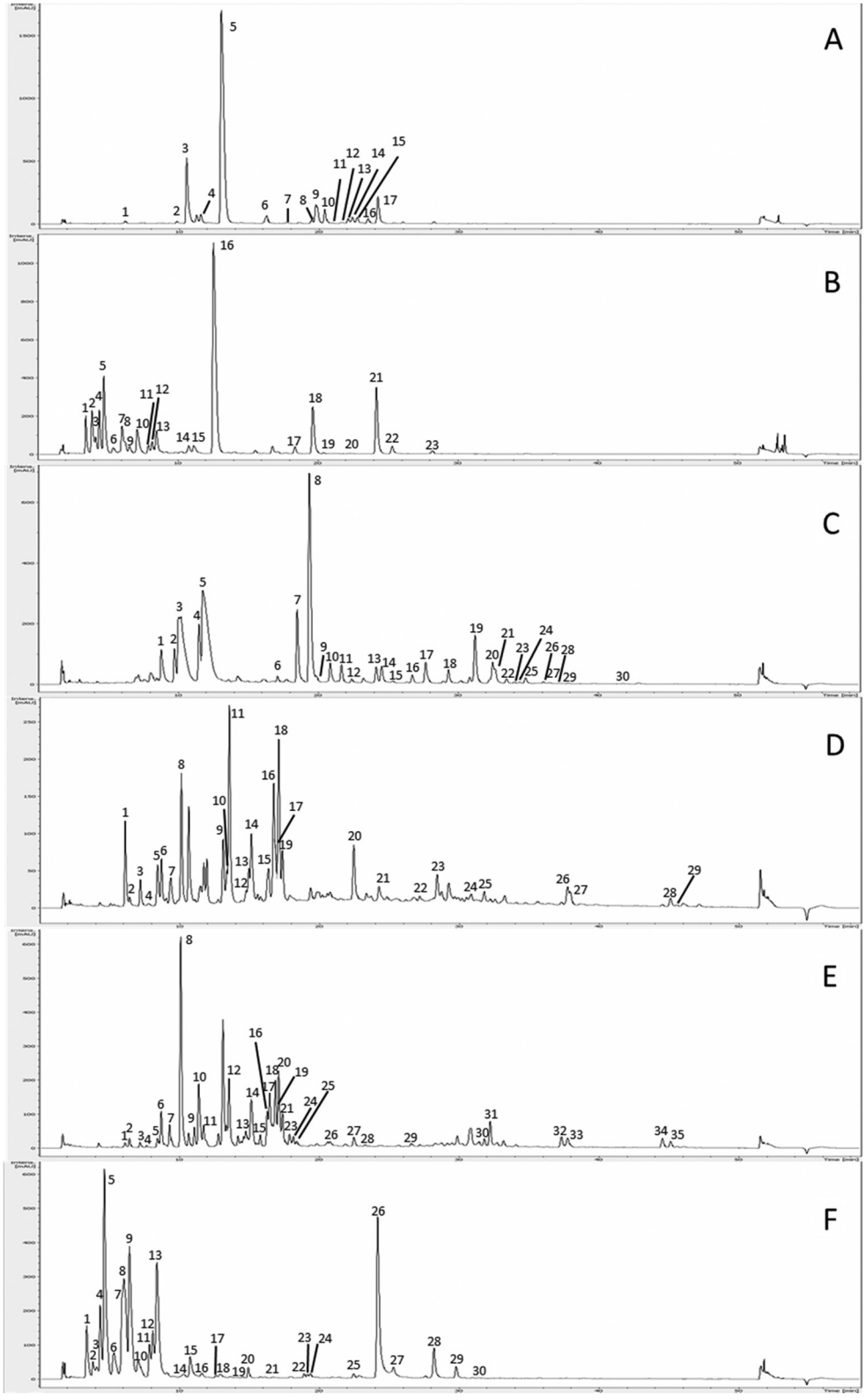

Fig. 1. Chromatograms of African nightshade (A), amaranth (B), Cowpea (C), common kale (D), Ethiopian kale (E), and spiderplant (F) at 330 nm. For the compounds referring to the peak numbers please see Tables 1 to 6.

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4500 rpm for 5 min until colorless. The combined supernatants were evaporated in a stream of nitrogen. The extracts were dissolved in 0.02 ml dichloromethane and 0.18 ml of isopropyl alcohol. Prior analysis the solutions were filtered through a 0.2 μM PTFE membrane and kept at 4 °C in the auto sampler during the analysis process. The separation was performed on a C30-column (YMC Co. Ltd. Japan, YMC C30, 100 × 2.1 mm, 3 μm) on an Agilent Technologies 1290 Infinity UHPLC equipped with a diode array detector. Mixtures of methanol, methyl-tert-butyl-ether and water in different volume ratios (solvent A: 81/15/4 and solvent B: 6/90/4, both 20 mM ammonium acetate) were used as mobile phases at a flow rate of 0.2 ml min1. Identification was achieved by co-chromatography with authentic references substances. External standard calibration curves were used for quantification by dose–response curves. In addition; the identity of the pigments was confirmed using an Agilent Technologies 6230 TOF LC/MS APCI ion source in positive ionization mode. The gas temperature was set to 325 °C at a flow rate of 8 L min-1, the vaporizer to 350 °C and the nebulizer pressure was set to 35 psi. The voltage was set to 3500 V and a fragmentor voltage of 175 V was applied at a corona current of 6.5 μA. (and analyzed on an Agilent Technologies 1290 Infinity UHPLC coupled with an Agilent Technologies 6230 QTOF LC/MS as described by Errard et al. (2015) and Mageney, Baldermann, and Albach (2016).

2.4. Glucosinolate analysis

The glucosinolate composition of the samples was determined as desulfo-glucosinolates, using a slightly modified method according to (Wiesner, Zrenner, Krumbein, Glatt, & Schreiner, 2013). The modifications were as follows: The various desulfo-glucosinolates were separated by a UHPLC-DAD device (UHPLC Agilent 1290 Infinity System, Agilent Technologies, Böblingen, Germany) equipped with a Poroshell 120 EC-C18 column of dimension 100 mm × 2.1 mm containing particles of size 2.7 μm (Agilent Technologies). The solvent gradient was formed by water (A) and 40% acetonitrile (B), starting at 0.5% B for 2 min, rising to 49.5% B over the next 10 min, then held for a further 2 min, increased to 99.5% B over the course of 1 min and finally held for a final 2 min. The flow rate was 0.4 mL min − 1 and the injection volume 5 μL. Desulfo-glucosinolates were identified by comparing retention times and UV absorption spectra with those of known standards. Quantification was done at 229 nm using the response factor of the glucosinolates relative to 2-propenyl glucosinolate (external standard). Determination of glucosinolates was performed in duplicate.

3. Results and discussion

In the investigated indigenous African leafy vegetables phenolic flavonoid and hyroxycinnamic acids, carotenoids, chlorophylls and glucosinolates were qualified and quantified. All investigated secondary plant metabolites were found to have species-specific modifications in both concentration and composition, and especially the flavonoids in their structural diversity. However, there are enormous differences in concentrations of the quantified carotenoids and glucosinolates. All secondary plant metabolite groups measured may have an impact on nutritional or health-promoting value of these indigenous African leafy vegetables.

3.1. Flavonoids and hydroxycinnamic acids

Indigenous African leafy vegetables are sources of various hydroxycinnamic acid derivatives and flavonoid glycosides (Fig. 1 and Tables 1–6). These compounds were identified by maximum absorption wavelength, retention time and molecular masses and fragmentation patterns. For quantification, external calibration curves for semi-quantitative analysis have been used as described above.

High concentrations of phenolic compounds were found in African nightshade (16,677 and 16,387 μg/g DW), Ethiopian kale (17,206 and 12,228 μg/g DW) and cowpea (16,185 and 17,408 μg/g DW) followed by medium concentrations in amaranth (14,221 and 9229 μg/g DW) and common kale (6690 and 12,143 μg/g DW). Low concentrations of phenolics occur in spiderplant (5297 and 6295 μg/g DW). In more detail, high concentrations of caffeic acid derivatives were identified in African nightshade (11,593 and 12,444 μg/g DW) and amaranth (9191 and 5702 μg/g DW) while the other investigated indigenous African leafy vegetables had low concentrations (<1000 μg/g DW). The concentration of quercetin glycosides was high in cowpea (10,602 and 11,888 μg/g DW), medium in African nightshade (4391 and 3268 μg/g DW), spiderplant (3084 and 3730 μg/g DW) and amaranth (2805 and 1692 μg/g DW) and low in common kale (191 and 352 μg/g DW) and Ethiopian kale (560 and 352 μg/g DW). This is comparable to data in literature (Hertog, Hollman, & Katan, 1992).

Moreover, the structure of hydroxycinnamic acids and flavonoid glycosides of the indigenous African leafy vegetables was investigated in more detail.

African nightshade (*S. scabrum*) is used as vegetable and medical plant (Manoko, van den Berg, Feron, van der Weerden, & Mariani, 2008). It is a relative of tomato (*S. lycopersicum*), potato (*S. tuberosum*)

Table 1
Phenolic compounds of African nightshade in μg/g DW.

						Nightshade 1	Nightshade 2
Tentative structure	No.	RT [min]	MS	MS^2	MS^3	Mean ± standard deviation	Mean ± standard deviation
3-Caffeoylquinic acid	1	6.19	353	191,179	126	125 ± 2.05	128 ± 0.46
5-Caffeoylquinic acid	2	9.89	353	173	111	104 ± 1.65	114 ± 1.29
4-Caffeoylquinic acid (dimer)	3	10.59	707	353	191	1942 ± 32.60	2204 ± 18.34
Caffeoylmalate isomer 1	4	11.6	295	179	135	249 ± 2.81	214 ± 6.12
Caffeoylmalate isomer 2 (dimer)	5	13.06	591	295	179,133	9173 ± 158.76	9784 ± 61.70
Quercetin-3-glucosylrhamnogalcatoside	6	16.31	771	301	179,151	527 ± 8.90	587 ± 9.95
Coumaric acid	7	17.76	163	118		104 ± 1.29	107 ± 0.66
Quercetin-3-rhamnogalactoside	8	19.53	625	301	179,151	138 ± 4.16	158 ± 2.62
Quercetin-3-rhamnosylrhamnogalactoside isomer 1	9	19.81	755	301,591	271,255,179,151	1374 ± 26.07	195 ± 4.68
Quercetin-3-rhamnosylrhamnogalactoside isomer 2	10	20.45	755	301,591	271,255,179,151	694 ± 12.40	655 ± 7.17
Sinapoylmalate	11	21.01	339	223	164	62 ± 2.76	61 ± 2.05
Kaempferol-3-diglucoside	12	21.64	609	285		86 ± 1.03	91 ± 1.09
Kaempferol-3-rhamnosylrhamnogalactoside isomer 1	13	22.13	739	285	255	120 ± 0.91	104 ± 0.49
Quercetin-3-pentosylrutinoside	14	22.44	741	301,609	271,255,179,151	251 ± 2.31	252 ± 3.15
Sinapic acid	15	23.13	223	205	164	60 ± 1.94	58 ± 0.32
Kaempferol-3-rhamnosylrhamnogalactoside isomer 2	16	23.55	739	575	285	261 ± 6.75	254 ± 5.80
Quercetin-3-rutinoside	17	24.26	609	301	179,151	1407 ± 252.30	1421 ± 15.02
Caffeic acid derivatives						**11,593**	**12,444**
Quercetin glycosides						**4391**	**3268**
Total phenols						**16,677**	**16,387**

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Table 2
Phenolic compounds of amaranth in μg/g DW.

						Amaranth 1	Amaranth 2
Tentative structure	No.	RT [min]	MS	MS^2	MS^3	mean ± standard deviation	mean ± standard deviation
Caffeoylglucaric isomer 1	1	3.48	371	209,173,191	191	520 ± 29.31	335 ± 8.47
Caffeoylglucaric isomer 2	2	3.92	371	209,173,191	191	539 ± 18.68	379 ± 6.92
Caffeoylglucaric isomer 3 (dimer)	3	4.18	743	371	209,173,191	123 ± 1.69	95 ± 0.63
Caffeoylglucaric isomer 4	4	4.45	371	209,173,191	191	541 ± 4.77	366 ± 2.22
Caffeoylglucaric isomer 5(dimer)	5	4.77	743	371	209,173,191	1258 ± 13.81	843 ± 4.49
Coumaroylglucaric isomer 1	6	5.51	355	191,209	147	211 ± 1.41	225 ± 1.37
Caffeoylglucaric isomer 6 (dimer)	7	6.1	743	371	209,173,191	673 ± 8.07	530 ± 3.84
Coumaroylglucaric isomer 2	8	6.41	355	191,209	147	49 ± 0.00	53 ± 0.00
Coumaroylglucaric isomer 3	9	6.6	355	191,209	147	177 ± 1.02	183 ± 0.62
Feruloylglucaric isomer 1	10	7.16	385	191,209	147,129	620 ± 6.23	467 ± 2.40
Feruloylglucaric isomer 2	11	7.96	385	191,209	147,129	147 ± 0.80	122 ± 0.67
Feruloylglucaric isomer 3	12	8.21	385	191,209	147,129	160 ± 1.15	118 ± 0.61
Feruloylglucaric isomer 4	13	8.52	385	191,209	147,129	387 ± 3.40	310 ± 1.94
Feruloylglucaric isomer 5	14	10.84	385	191,209	147,129	179 ± 1.64	147 ± 1.21
Caffeoyl isocitrat isomer 1	15	11.2	353	173,191	155,111	212 ± 0.81	134 ± 1.30
Caffeoyl isocitrat isomer 2	16	12.6	707	353	173,191	5325 ± 48.54	3020 ± 23.59
Feruloyl isocitrat isomer 1	17	18.46	367	173,191	155,111	157 ± 66.85	101 ± 0.29
Feruloyl isocitrat isomer 2	18	19.7	367	173,191	155,111	510 ± 0.97	370 ± 1.36
Quercetin-3-rutinoside-7-rhamnoside	19	20.47	755	301,591	271,255,179,151	26 ± 0.62	23 ± 0.55
Quercetin-7-rutinoside	20	22.3	609	301	271,255,179,151	52 ± 0.36	51 ± 0.31
Quercetin-3-rutinoside	21	24.24	609	301	179,151,271,255	2448 ± 38.97	1464 ± 12.42
Quercetin-3-glucoside	22	25.36	463	301	179,151,271,255	279 ± 2.47	154 ± 6.60
Kaempferol-3-rutinoside	23	28.24	593	285	255	87 ± 0.72	72 ± 1.08
Caffeic acid derivatives						**9191**	**5702**
Quercetin glycosides						**2805**	**1692**
Total phenols						**14,221**	**9229**

or eggplant (*S. melongena*). African nightshade is characterized by high concentrations of hydroxycinnamic acid derivatives especially caffeoylmalates and caffeoylquinic acids (Table 1). These have previously been described in *S. melongena* (Meyer et al., 2015) and *S. lycopersicum* (Cle et al., 2008). While chlorogenic acid (5-caffeoylquinic aicd) is a well-known antioxidant of coffee (Buscemi et al., 2016) the bioactivity of caffeoylmalate is not yet investigated. Medium concentrations of flavonoids are found in African nightshade. However, these are

Table 3
Phenolic compounds of cowpea in μg/g DW.

						Cowpea 1	Cowpea 2
Tetative structure	No.	RT [min]	MS	MS^2	MS^3	Mean ± standard deviation	Mean ± standard deviation
Coumaric acid (dimer)	1	8.83	325	163	119	445 ± 15.74	367 ± 24.17
unknown	2	9.78	341	195	129,177	404 ± 3.24	315 ± 3.12
Quercetin-3-soph-7-glc	3	10.08	787	625	301	1490 ± 1384.04	2687 ± 27.80
Quercetin-3-arabinosyl-diglc	4	11.53	757	595	300	42 ± 5.83	27 ± 1.29
Coumaric acid isomer 2	5	11.81	163	119		3209 ± 31.87	3394 ± 17.87
Quercetin-3-glc-7-glc	6	17.16	625	463	301	118 ± 6.05	113 ± 0.38
Quercetin-3- arabinosyl-diglc	7	18.56	757	300.625		1536 ± 25.40	1563 ± 10.09
Quercetin-7-diglc	8	19.42	625.1251	625	300	5322 ± 84.09	5338 ± 106.27
Quercetin-3-diglc	9	19.93	625	301	179,151	108 ± 1.91	74 ± 39.79
Kaempferol-3-arabinosyl-diglc	10	20.92	741	285	254	325 ± 6.96	264 ± 1.86
Kaempferol-3-soph	11	21.72	609	285	254	257 ± 12.63	262 ± 2.20
Isorhamnetin-3-arabinosyl-diglc	12	22.45	771	315,621		162 ± 2.55	133 ± 1.30
Isorhamnetin-3-diglc	13	24.2	639	315	300	458 ± 6.42	453 ± 7.27
Quercetin-7-glc	14	24.59	463	301	179,151	351 ± 9.36	256 ± 46.89
Quercetin-3-glc	15	25.39	463	301	179,151	59 ± 0.57	51 ± 11.31
Quercetin-3-coumaroyl-arabinosyl-diglc	16	26.79	903	757	301,343	164 ± 1.38	193 ± 1.16
Quercetin-3-coumaroyl arabinosyl-diglc	17	27.73	903	757	301,343	351 ± 6.59	387 ± 14.35
Quercetin-3-acetyl-glc	18	28.99	505	301,463	179,151	56 ± 0.44	51 ± 8.55
Quercetin-3-coumaroyl-diglc	19	31.26	771	625	301	663 ± 13.26	769 ± 16.39
Quercetin-3-feruloyl-diglc	20	32.53	801	625	301	87 ± 4.24	86 ± 1.13
Quercetin-3-coumaroyl-diglc	21	32.71	771	625	301	111 ± 2.71	141 ± 1.00
Kaempferol-3-coumaroyl-arabinosyl-diglc	22	33.51	755	609	285	70 ± 1.77	76 ± 1.55
Quercetin-3-feruloyl-diglc	23	34.07	801	625	301	45 ± 1.06	49 ± 0.33
Kaempferol-3-coumaroyl-diglc	24	34.45	755	609	285	48 ± 0.17	49 ± 0.51
Isorhamnetin-3-coumaroyl-diglc	25	34.87	785	639	315,3	97 ± 0.98	103 ± 1.01
Isorhamnetin-3-feruloyl-diglc	26	36.14	815,837	639	315	52 ± 0.75	43 ± 0.90
Isorhamnetin-3-coumaroyl-diglc	27	36.58	785	639	315,3	56 ± 1.49	61 ± 0.91
Quercetin-3-coumaroyl-rutinoside	28	37.2	755	607	301	28 ± 3.01	26 ± 0.71
Quercetin-3-coumaroyl-diglc	29	37.94	771	625	301,463	34 ± 0.65	43 ± 0.47
Quercetin-3-coumaroyl-rutinoside	30	41.86	755	609	301	37 ± 1.08	34 ± 4.03
Caffeic acid derivatives						**0**	**0**
Quercetin glycosides						**10,602**	**11,888**
Total phenols						**16,185**	**17,408**

Soph: sophoroside, glc: glucoside.

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Table 4
Phenolic compounds of common kale in μg/g DW.

						Common kale 1	Common kale 2
Tentative structure	No.	RT [min]	MS	MS^2	MS^3	Mean ± standard deviation	Mean ± standard deviation
Caffeoylquinic acid (chlorogenic acid)	1	6.2	353	191		326 ± 3.83	792 ± 4.77
Caffeoyl-glycoside	2	6.5	341	179, 161	165	62 ± 0.41	93 ± 0.69
3-*p*-Coumarolyquinic acid	3	7.2	335	191		196 ± 2.68	448 ± 4.46
Hydroxyferuloyl-glycoside	4	7.8	371	209, 191		62 ± 0.15	56 ± 0.40
5-*p*-Coumaroylquinic acid	5	8.5	335	191		250 ± 3.62	648 ± 3.66
Quercetin-3-O-soph-7-O-glc	6	8.8	787	625	301,463,	283 ± 2.62	766 ± 2.59
p-Coumaroyl-glycoside	7	9.4	325	163, 145		167 ± 1.84	284 ± 0.49
Kaempferol-3-O-soph-7-O-glc	8	10.2	771	609	285,429	517 ± 7.34	1280 ± 6.74
Kaempferol-3-O-hydroxyferuoyl-soph-7-O-diglc	9	13.2	1125	801	609	351 ± 5.21	1112 ± 9.18
Kaempferol-3-O-hydroxyferuoyl-soph-7-O-glc	10	13.4	963	801	609	65 ± 1.38	41 ± 0.25
Kaempferol-3-O-caffeoyl-soph-7-O-glc	11	13.6	933	771	609	990 ± 13.85	2371 ± 11.81
Quercetin-3-O-soph-7-O-sinapoyl-diglc	12	14.5	1155	949	625	30 ± 0.64	34 ± 0.45
Quercetin-3-O-soph-7-O-feruloyl-diglc	13	14.8	1125	949	625	41 ± 0.63	85 ± 1.04
Quercetin-3-O-sinapoyl-soph-7-O-glc	14	15.0	993	831	625	82 ± 1.16	89 ± 0.56
Kaempferol-3-O-sinapoyl-sophoroside-7-O-diglc	15	16.4	1139	815	609	268 ± 3.89	539 ± 3.68
Kaempferol-3-O-feruloyl-soph-7-O-diglc	16	16.8	1109	785	609	690 ± 10.53	334 ± 10.08
Kaempferol-3-O-sinapoyl-soph-7-O-glc	17	17.0	977	815	609	50 ± 4.65	235 ± 9.05
Kaempferol-3-O-feruloyl-soph-7-O-glc	18	17.1	947	785	609	766 ± 10.96	1266 ± 9.03
Kaempferol-3-O-coumaroyl-soph-7-O-glc	19	17.4	917	755	609	205 ± 1.93	374 ± 2.99
Kaempferol-3-O-hydroxyferuoyl-soph	20	22.5	801	609	285,429	432 ± 6.99	350 ± 5.91
Kaempferol-3-O-caffeoyl-soph	21	24.3	771	609	285,429	161 ± 1.83	88 ± 1.00
Kaempferol-3-O-sinapoyl-soph	22	26.8	815	609	285,429	76 ± 0.36	112 ± 0.69
Kaempferol-3-O-feruloyl-soph	23	28.5	785	609	285,429	184 ± 2.03	74 ± 0.81
Quercetin-3-O-disinapoyl-triglc-7-O-D-glc	24	30.6	1361	1199	993	38 ± 0.22	35 ± 0.37
Kaempferol-3-O-disinapoyl-triglc-7-O-D-glc	25	31.8	1345	1183	977	105 ± 1.21	154 ± 1.66
Disinapoyl-gentiobioside	26	37.8	753	529	223,288	86 ± 0.30	193 ± 2.56
Sinapoyl-feruloyl-gentiobiose	27	38.0	723	499	193,258	64 ± 0.20	80 ± 1.31
Trisinapoyl-gentiobioside	28	45.2	959	735	529,223	89 ± 0.36	154 ± 1.12
Disinapoyl-feruloyl-gentiobiose	29	45.6	929	705	529,223	54 ± 0.05	56 ± 0.29
Caffeic acid derivatives						**388**	**885**
Quercetin glycosides						**191**	**243**
Total phenols						**6690**	**12,143**

Soph: sophoroside, glc: glucoside.

mainly quercetin glycosides characterized by the loss of 454 Da in the MS^2. Potawale et al. (2008)) describes quercetin-3-glucoyslrhamnosylgalactoside and quercetin-3-rhamnosylgalactoside in *S. nigrum* which is in agreement with the present data on *S. scabrum*. The main compound is quercetin-3-rutinoside (rutin) which is also the main flavonoid glycoside in *S. lycopersicum* (Cle et al., 2008) and is studied for its antioxidant, anti-inflammatory, anti-allergy, and anti-tumor activity (Habtemariam, 2016; Hosseinzadeh & Nassiri-Asl, 2014; Koval'skii et al., 2014). The other identified quercetin glycosides are seldom and not yet studied but might also have an effect on the antioxidant activity of African nightshade.

Amaranth (*A. cruentus*) leaves are consumed as vegetable in Eastern Africa because it is fast growing and available most of the year (Rastogi & Shukla, 2013). Although *Chenopodium quinoa* also used as pseudo-cereal had a comparable total phenolic content, the antioxidant activity of amaranth was higher (Nsimba, Kikuzaki, & Konishi, 2008). Consequently, the leaves of amaranth are of interest due to their hydroxycinnamic derivatives with very high concentrations of caffeoylglucaric isomers and caffeoyl isocitrate isomers (Table 2). Hydroxycinnamic acid glucaric isomers are tentative identified by their deprotonated molecular ions *m/z* 355 for coumaroyl, *m/z* 371 for caffeoyl and *m/z* 385 for feruloyl. In the MS^2 the glucaric acid moiety is represented by fragments with *m/z* 209 and *m/z* 191 after loss of the hydroxycinnamic acid residue 146 Da for coumaroyl, 162 Da for caffeoyl and 176 Da for feruloyl. Glucaric acids of coumaroyl, caffeoyl and feruloyl have been previously identified by HPLC-MS in pineapple (*Ananas comosus*) (Steingass, Glock, Schweiggert, & Carle, 2015), dog's mercury (*Mercurialis perennis*) (Lorenz et al., 2012) and calafate (*Berberis microphylla*) (Ruiz et al., 2014). For caffeoyl glucaric acids in calafate first preliminary NMR data gave 3 and 4-trans-caffeoyl glucaric acids (Ruiz et al., 2014; Strack, Gross, Wray, & Grotjahn, 1987). The hydroxycinnamic acid isocitrates are characterized by the deprotonated molecular ions *m/z* 353 for caffeoyl isocitrat isomers and *m/z* 367 for feruloyl isocitrat isomers the fragments in the MS^2 are *m/z* 191 and *m/z* 173. In *A. cruentus* previously caffeoyl isocitrat isomers have been identified by NMR (Strack, Leicht, Bokern, Wray, & Grotjahn, 1987). Neither the hydroxycinnamic acid glucaric isomers nor the hydroxycinnamic acid isocitrate isomers have been described in nutritional plants before or are investigated on their health-promoting potential so far. Flavonoid glycosides are present in amaranth in medium concentrations of 2805 and 1692 μg/g DW quercetin glycosides compared the other investigated species with up to 10,602 and 11,888 μg/g DW quercetin glycosides in cowpea. Mainly quercetin glycosides were tentatively identified with quercetin-3-rutinoside (rutin) as main compound followed by kaempferol-3-rutinoside (nicotiflorin) a minor flavonoid glycoside in amaranth. Both compounds have been previously described in different amaranth species (Barba de la Rosa et al., 2009; Niveyro, Mortensen, Fomsgaard, & Salvo, 2013; Steffensen, Pedersen, Labouriau, Mortensen, Laursen, de Troiani, Noellemeyer, Janovska, Stavelikova, Taberner, Christophersen and Fomsgaard, 2011; Steffensen, Rinnan, Mortensen, Laursen, Troiani, Noellemeyer, Janovska, Dusek, Delano-Frier, Taberner, Christophersen and Fomsgaard, 2011). Additionally, quercetin-3-glucoside (isoquercetrin) has been determined in amaranth (Barba de la Rosa et al., 2009; Khanam & Oba, 2013; Khanam, Oba, Yanase, & Murakami, 2012; Niveyro et al., 2013; Steffensen, Pedersen, et al., 2011; Steffensen, Rinnan, et al., 2011). Consequently, the quercetin to kaempferol ratio of amaranth is higher than 10 which is of high interest for human nutrition due to the antioxidant activity expected from quercetin glycosides (Zietz et al., 2010). Due to its high concentration of secondary plant metabolites including rutin, *A. spinosus,* a species related to *A. cruentus,* is a medical plant with pharmacological actions against diabetes, cancer, and inflammation. Additionally, it has hepato-protective, anti-malarial, and anti-oxidative properties (Kumar, Jindal, Gupta, & Rana, 2014).

Cowpea (*V. unguiculata*) has a high antioxidant activity (Chon, 2013; Zia-Ul-Haq, Ahmad, Amarowicz, & De Feo, 2013). It is however

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Table 5
Phenolic compounds of Ethiopian kale in μg/g DW.

						Ethopian kale 1	Ethopian kale 2
Tentative structure	No.	RT [min]	MS	MS^2	MS^3	Mean ± standard deviation	Mean ± standard deviation
Caffeoylquinic acid (chlorogenic acid)	1	6.5	353	191		109 ± 0.97	74 ± 0.46
Caffeoyl-glycoside	2	6.71	341	179, 161	135	114 ± 0.30	94 ± 0.49
3-*p*-Coumarolyquinic acid	3	7.22	337	163	118	62 ± 0.28	53 ± 0.04
Hydroxyferuloyl-glycoside	4	7.91	371	209, 191	194,164,150	52 ± 0.16	52 ± 0.11
5-*p*-Coumaroylquinic acid	5	8.5	337	163	119,0	125 ± 0.77	94 ± 0.98
Quercetin-3-O-soph-7-O-D-glc	6	8.77	787	625	301,463,445	466 ± 1.92	292 ± 4.18
p-Coumaroyl-glycoside	7	9.37	325	163, 145	119,0	244 ± 1.40	125 ± 0.68
Kaempferol-3-O-soph-7-O-glc	8	10.16	771	609	285,429	1849 ± 13.80	1206 ± 15.53
Kaempferol-3-O-soph-7-O-diglc	9	11.13	933	609	285	153 ± 0.94	118 ± 0.90
Isorhamnetin-3-O-glc-7-O-glc	10	11.45	639	477	315	3044 ± 22.92	1975 ± 25.79
Kaempferol-3-O-hydroxyferuoyl-soph-7-O-glc	11	13.19	963	801	609	1411 ± 7.86	877 ± 14.17
Kaempferol-3-O-caffeoyl-soph-7-O-glc	12	13.62	933	771	609	660 ± 3.93	459 ± 4.55
Isorhamnetin-3-O-hydroxyferuloyl-soph-7-O-glc	13	14.25	993	831	639,315	2316 ± 13.06	1428 ± 23.54
Isorhamnetin-3-O-caffeoyl-soph-7-O-diglc	14	14.66	963	801	315	1069 ± 6.53	735 ± 7.56
Isorhamnetin-3-O-hydroxyferuloyl-soph-7-O-diglc	15	14.82	1155	993	639,315	178 ± 1.22	130 ± 2.50
Kaempferol-3-O-glc-7-O-glc	16	15.2	609	447	285	522 ± 3.30	359 ± 4.62
Kaempferol-3-O-sinapoyl-soph-7-O-diglc	17	16.35	1139	815	609	183 ± 0.95	135 ± 2.97
Kaempferol-3-O-feruloyl-soph-7-O-diglc	18	16.53	1109	785	609	332 ± 2.57	235 ± 2.32
Isorhamnetin-3-O-caffeoyl-soph-7-O-glc	19	16.93	801	639	315	277 ± 1.58	197 ± 4.93
Kaempferol-3-O-sinapoyl-soph-7-O-glc	20	17.17	977	815	609	666 ± 5.22	520 ± 6.18
Kaempferol-3-O-feruloyl-soph-7-O-glc	21	17.43	947	785	609	267 ± 1.77	190 ± 2.55
Kaempferol-3-O-coumaroyl-soph-7-O-glc	22	17.94	917	755	609	151 ± 1.90	120 ± 2.52
Isorhamnetin-3-O-sinapoyl-sophoroside-7-O-glucoside	23	18.21	1007	845	639,315	416 ± 2.94	288 ± 4.24
Isorhamnetin-3-O-feruloyl-soph-7-O-glc	24	18.45	977	815	639,315	223 ± 3.15	172 ± 4.19
Isorhamnetin-3-O-coumaroyl-soph-7-O-glc	25	18.94	947	785	639,315	151 ± 1.16	130 ± 1.66
Isorhamnetin-3-O-soph	26	21.54	639	315,447	300	43 ± 0.53	55 ± 0.36
Kaempferol-3-O-soph	27	22.54	609	285	255	840 ± 5.48	569 ± 7.68
Isorhamnetin-3-O-soph	28	23.34	639	315,459	300	200 ± 2.59	821 ± 7.97
Kaempferol-3-O-sinapoyl-soph	29	28.5	785	609	285	62 ± 2.61	41 ± 0.16
Quercetin-3-O-disinapoyl-triglc-7-O-glc	30	31.48	1361	1199	993	94 ± 0.61	60 ± 0.65
Kaempferol-3-O-disinapoyl-triglc-7-O-glc	31	32.28	1345	1183	977	371 ± 0.76	225 ± 3.21
Disinapoyl-gentiobioside	32	37.37	753	529	223,288	144 ± 0.48	103 ± 0.98
Sinapoyl-feruloyl-gentiobiose	33	37.81	723	499	193,258	148 ± 0.33	108 ± 0.79
Trisinapoyl-gentiobioside	34	44.59	959	735	529,223	152 ± 0.85	104 ± 1.03
Disinapoyl-feruloyl-gentiobiose	35	45.17	929	705	529,223	112 ± 0.07	84 ± 0.67
Caffeic acid derivatives						**223**	**168**
Quercetin glycosides						**560**	**352**
Total phenols						**5297**	**6295**

Soph: sophoroside, glc: glucoside.

characterized by low concentrations of hydroxycinnamic acid derivatives mainly coumaroyl derivatives with coumaric acid as main compound (Table 3). Coumaric acid has also been described in *V. sinensis* (Duenas, Fernandez, Hernandez, Estrella, & Munoz, 2005; Nderitu, Dykes, Awika, Minnaar, & Duodu, 2013). In cowpea non-acylated, mono- and diglucosides of quercetin, kaempferol and isorhamnetin are tentatively identified. Quercetin and kaempferol mono- and digycosides with glucose and galactose as sugar moieties have been previously identified in *V. sinensis* by HPLC (Nderitu et al., 2013) and by NMR (Cui et al., 2012). A high number of structurally different coumaroyl and feruloyl monoacylated quercetin, kaempferol and isorhamnetin glycosides were found, and arabinosyl-diglucosides of quercetin, kaempferol and isorhamnetin were also determined. The loss of 456 Da in the MS^2 represents the loss of the arabiosyl-diglucoside moiety giving rise to the flavonoid aglycone ions *m/z* 301, *m/z* 285 and *m/z* 315 for quercetin, kaempferol and isorhamnetin, respectively. A quercetin-3-arabiosyl-diglucoside has been previously detected in other cowpea cultivars (Nderitu et al., 2013). The highest concentrations were found for non-acylated quercetin glycosides quercetin-3-sophoroside, quercetin-3-sophoroside-7-glucoside, and quercetin-arabinosyl-diglucoside. Flavonoid glycosides of cowpea are discussed to inhibit LDL (low density lipoprotein) oxidation and consequently, atherosclerosis (Cui et al., 2012) and DNA damage (Nderitu et al., 2013). The high antioxidant activity of cowpea (Chon, 2013; Zia-Ul-Haq et al., 2013) might therefore be related to the high concentration of quercetin glycosides found in cowpea.

Both common kale (*B. oleracea*) and Ethopian kale (*B. carinta*) belong to the same family Brassicaceae and genus *Brassica* but are different species. Consequently, they overlap in their hydroxycinnamic acid and flavonoid glycoside profiles but show species-specific differences. Both are leafy vegetables eaten in East Africa (Olwande, Smale, Mathenge, Place, & Mithofer, 2015; Sharma, Kalia, Yadava, Singh, & Sharma, 2016). In common kale the main hydroxycinnamic acid derivative is caffeoylquinic acid (3-chlorogenic acid) (Table 4). Other relevant hydroxycinnamic acids are gentiobiosides of sinapoyl and feruloyl. The main flavonoid glycosides are monoacylated kaempferol di-, tri- and tetraglycosides such as kaempferol-3-*O*-sinapoyl-sophoroside-7-*O*-diglucoside. Common kale also contains various quercetin glycosides including monoacylated quercetin triglucoside quercetin-3-*O*-sinapoyl-sophoroside-7-*O*-glucoside. The fragmentation pattern corresponds to that of kaempferol glycosides (Schmidt et al., 2010). The main flavonoid glycosides of kale have been previously identified by NMR (Fiol et al., 2012). These present results are in line with former results on kale flavonoids (Ferioli et al., 2013; Ferreres et al., 2009; Lin & Harnly, 2009; Neugart et al., 2014; Olsen, Aaby, & Borge, 2009; Schmidt et al., 2010). Kale extracts inhibit growth and induce apoptosis in different colon cancer cells (Olsen, Grimmer, Aaby, Saha, & Borge, 2012). However, Fiol et al. (2012) showed that complex acylated quercetin glycosides from kale have higher radical scavenging activity than their corresponding kaempferol glycosides and are therefore of higher interest for human nutrition as antioxidants. In Ethiopian kale the main hydroxycinnamic acid is coumaroyl-glucoside and a higher number of kaemperol and isorhamnetin diglycosides have been characterized (Table 5). Also a high number of monoacylated kaempferol tri- and tetraglycosides could be tentatively identified. However, the main flavonoid glycosides are monoacylated iosrhamnetin tri- and tetraglycosides. Up to date, no

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Table 6
Phenolic compounds of spiderplant in μg/g DW.

						Spider plant 1	Spider plant 2
Tentative structure	No.	RT [min]	MS	MS^2	MS^3	Mean ± standard deviation	Mean ± standard deviation
Caffeoylglucaric isomer 1 (dimer)	1	3.43	743	371	209	89 ± 0.89	84 ± 16.09
Caffeoylglucaric isomer 2 (dimer)	2	3.88	743	371	209	57 ± 0.57	55 ± 0.23
Caffeoylglucaric isomer 3	3	4.16	371	209	191	52 ± 0.97	52 ± 0.86
Caffeoylglucaric isomer 4 (dimer)	4	4.39	743	371	209	83 ± 0.61	81 ± 13.61
Caffeoylglucaric isomer 5 (dimer)	5	4.7	743	371	209	20 ± 0.06	175 ± 45.90
Coumaroylglucaric isomer 1	6	5.51	355	191, 209		55 ± 1.51	53 ± 1.86
Coumaroylglucaric isomer 2	7	6	355	191, 209		78 ± 0.71	79 ± 14.87
Coumaroylglucaric derivative	8	6.23	727	355	191, 209	57 ± 0.13	55 ± 0.72
Coumaroylglucaric isomer 3 (dimer)	9	6.51	711	355	191, 209	64 ± 0.48	64 ± 0.58
Feruloylglucaric isomer 1	10	7.11	385	191	147,127	69 ± 0.16	66 ± 2.45
Coumaroylglucaric isomer 4	11	7.92	355	191, 209		61 ± 0.43	58 ± 4.04
Feruloylglucaric isomer 2	12	8.16	385	191	147,129	63 ± 0.10	62 ± 2.03
Feruloylglucaric isomer 3 (dimer)	13	8.47	771	385	191	124 ± 1.32	113 ± 9.62
Feruloylglucaric isomer 4	14	10.34	385	191	146,173	54 ± 0.48	52 ± 3.66
Feruloylglucaric isomer 5	15	10.83	385	191	146,173	75 ± 0.17	75 ± 9.87
unknown	16	11.99	593	473	353	94 ± 0.02	83 ± 1.66
Quercetin-3-diglucoside	17	12.68	625	301	179,151	52 ± 0.44	50 ± 17.85
Quercetin-3-rutinoside-7-glucoside	18	12.99	771	609	301	79 ± 4.48	75 ± 2.79
Aglycone-3-rutinoside + 89	19	13.97	710	621	313,284	41 ± 0.07	38 ± 2.30
Aglycone-3-rutinoside + 103	20	14.57	724	621	313,284	52 ± 0.14	65 ± 21.47
Quercetin-3-rutinoside + 66	21	16.73	673	609	301	46 ± 4.82	60 ± 25.04
Unknown	22	18.07	608	454	300	63 ± 0.50	77 ± 24.26
Unknown	23	18.98	563	443	535	61 ± 0.62	56 ± 4.41
Unknown	24	19.23	563	443	535	53 ± 0.04	57 ± 1.83
Kaempferol-3-diglucoside	25	22.51	609	429	285	83 ± 1.26	74 ± 8.05
Isorhamnetin-3-diglucoside	26	22.91	639	459	315	101 ± 1.62	93 ± 10.87
Quercetin-3-rutinoside	27	24.23	609	301	179,151	2747 ± 305.52	3332 ± 851.80
Quercetin-3-neohesperidoside	28	25.35	609	301	179,151	160 ± 6.63	213 ± 72.99
Kaempferol-3-rutinoside	29	28.26	593	285		307 ± 277.98	553 ± 39.11
Isorhamnetin-3-rutinoside	30	29.85	623	315	300	314 ± 4.10	303 ± 35.83
Isorhamnetin-3-glucoside	31	31.01	477	315	300	43 ± 43.16	42 ± 43.18
Caffeic acid derivatives						**301**	**447**
Quercetin glycosides						**3084**	**3730**
Total phenols						**5297**	**6295**

data on the identification of flavonoid glycosides in *B. carinata* exist. As *B. carinata* is an allotetrapolid derived from *B. oleracea* and *B. nigra* (Chang, Sun, Kakihara, & Hondo, 2015) these species can be used for identification of flavonoid glycosides and hydroxycinnamic acid derivatives. Different non-acylated and acylated kaempferol glycosides have been identified in various *B. oleracea* species (Ferioli et al., 2013; Ferreres et al., 2009; Ferreres et al., 2005; Lin & Harnly, 2009; Llorach, Gil-Izquierdo, Ferreres, & Tomás-Barberán, 2003; Neugart et al., 2014; Olsen et al., 2009; Schmidt et al., 2010; Vallejo, Tomás-Barberán, & Ferreres, 2004; Velasco et al., 2011). However, only Sun et al. (2013) and Schmidt et al. (2010) found in *Brassica* species monoacylated isorhamnetin glycosides comparable to the ones observed in *B. carinata*. The health-promoting effects of *B. carinata* have not yet been studied. Further research is needed as other species of the genus *Brassica* are highly discussed as healthy vegetables.

In leaves of spiderplant (*Cleome gynandra*) grown in East and South Africa (Wangolo, Onyango, Gachene, & Mong'are, 2015), hydroxycinnamic acid glucaric isomers are tentatively identified as previously described for amaranth (Table 6). These compounds have not been found in other nutritional plants so far. Spiderplant contains medium concentrations of quercetin, kaempferol and isorhamnetin diglycosides. However, the main flavonoid glycoside is quercetin-3-rutinoside (rutin). No literature exists on the identification of flavonoids aglycones or glycosides in *Cleome* species. Based on the retention time and mass spectra compared to the other indigenous African leafy vegetables these flavonoid glycosides have been tentatively identified. Spiderplant has a high antioxidant activity and is therefore of enormous interest for human nutrition and traditional medicine (Asis, Biswakant, Sagar, Haldar, & Majumder, 2009; Jansen, Gaba, & Greenberg, 1998; Kipandula, Mwanza, Nguu, & Ogoyi, 2014; Mibei, Ojijo, Karanja, & Kinyua, 2012; Srinivas, Chaitanya, Chandrashekar, & Bahavani, 2014).

3.2. Carotenoids

Indigenous African leafy vegetables are rich in carotenoids and chlorophylls (Fig. 2). Carotenoids and chlorophylls were identified by co-chromatography with authentic reference compounds based on maximum absorption wavelengths and molecular masses (Errard et al., 2015; Mageney et al., 2016; Mercadante, Egeland, Britton, Liaaen-Jensen, & Pfander, 2004). External standard calibration curves with the following qualifiers: β-carotene, $[M + H]^+$ 537.446; lutein, $[M - H_2O + H]^+$ 551.425; zeaxanthin, $[M + H]^+$ 569.435; violaxanthin, $[M + H]^+$ 601.452; neoxanthin, $[M - H_2O + H]^+$ 583.415; chlorophyll *a* $[M + H]^+$ 893.543, and Chlorophyll b $[M + H]^+$ 907.522 were used for quantification.

In the present study highest carotenoid concentrations (β-carotene, lutein, zeaxanthin, neoxanthin, and violaxanthin) were found in cowpea (843 and 918 μg/g DW) and spiderplant (772 and 853 μg/g DW). The concentrations were slightly lower in amaranth (682 and 704 μg/g DW) and common kale (553 and 626 μg/g DW) and lowest concentrations were determined in African nightshade (395 and 437 μg/g DW) and Ethiopian kale (222 and 325 μg/g DW). In general, green vegetables are rich in β-carotene, lutein, and zeaxanthin (Watzl & Leitzmann, 2005). β-Carotene, a pro-vitamin A active carotene, can be found for example in spinach up to 41 μg/g or kale up to 47 μg/g (Souci, Fachmann, & Kraut, 2008). In the present study in the common kale 22.8 and 24.8 μg/g DW β-carotene were found. Similar concentrations were determined in cowpeas (22.3 and 23.1 μg/g DW). The concentrations were lower in Ethiopian kale (4.9 and 6.4 μg/g DW) and African nightshade (7.1 and 7.9 μg/g DW). Better sources for pro-vitamin A are spiderplant (57.1 and 64.7 μg/g DW) and amaranth (47.1 and 101.7 μg/g DW). These concentrations are slightly lower compared to the previous study by Tang et al. (2014), where they determined β-carotene

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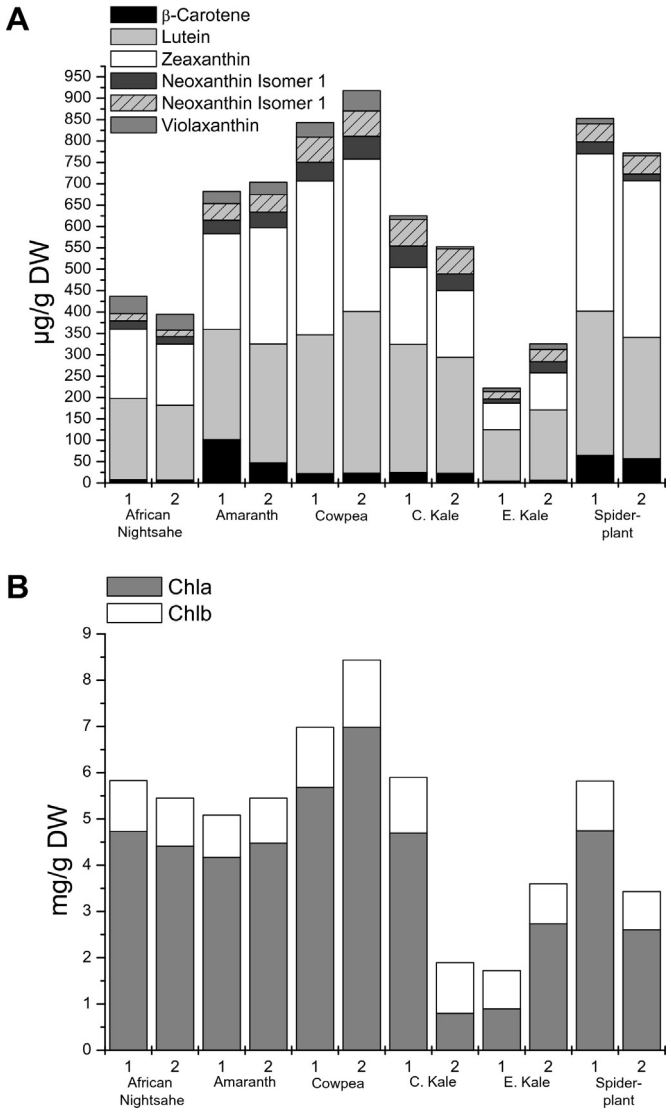

Fig. 2. Concentration of carotenoids (A) and chlorophylls (B) in indigenous African vegetables.

concentrations of 115.9 to 394.31 µg/g DW in amaranth leaves. These might be the result of different cultivars investigated in the two studies, cultivation areas or nutrient supply, e.g. nitrogen fertilization. However, the present study confirms that amaranth leaves are a good source for pro-vitamin A. Therefore, growing spiderplant and amaranth in home gardens complements dietary fortification and could contribute to better vitamin A status in Kenya.

Lutein and zeaxanthin are the major carotenoids in indigenous African leafy vegetables (Fig. 1A). Whereas the common kale and Ethiopian kale accumulate more lutein compared to zeaxanthin, the other indigenous African vegetables store more zeaxanthin. A previous study revealed that the concentrations of lutein and zeaxanthin strongly depend on the cultivar and growing conditions in kale (Mageney et al., 2016). Lutein and zeaxanthin are secondary plant metabolites with anti-oxidative properties. Moreover, they are major pigments in the human *macula lutea* and are known to reduce damage through absorbing blue light. Both xanthophylls have been related to protection against age-related macular degeneration (AMD) and cataracts. AMD is one of the main causes of blindness in developed countries which results in the loss of vision in the center of the visual field in elderly people (Renzi & Johnson, 2008). Therefore, indigenous African leafy vegetables could not only contribute in their country of origin to an improved diet, but also as extra vegetables or products in developed countries. In addition, all vegetables contain high amounts of neoxanthin and violaxanthin, where less is known about their biological activities in humans. Besides carotenes and xanthophylls the selected indigenous African leafy vegetables are a good dietary source for chlorophylls. It is discussed that a high uptake of chlorophylls (Fig. 1B) contributes strongly to the health beneficial properties associated with a plant based diet. For instance chorophyll b attenuates DDP-induced (cis-diamminedichloroplatinum) oxidative stress, chromosome instability, and lipid peroxidation in mice (Serpeloni et al., 2011).

3.3. Glucosinolates

Glucosinolates were identified by comparing retention times and absorption spectra with those of standard substances (Fig. 3). Quantification was done using the response factor of the glucosinolates relative to 2-propenyl glucosinolate as external standard.

In contrast to the ubiquitous occurring phenolics and carotenoids, glucosinolates are secondary plant metabolites limited to the order Brassicales. Consequently, glucosinolates were only identified in the *Brassica* and *Cleome* species of the investigated indigenous African leafy vegetables. The highest concentration of total glucosinolates was quantified in spiderplant (68 and 69 µmol/g DW) followed by Ethiopian kale (57 and 37 µmol/g DW) and common kale (12 and 32 µmol/g DW). The indigenous African leafy vegetables not only differ much in their total glucosinolate concentration, but also in their species-specific glucosinolate profiles.

Even if common kale (*B. oleracea*) and Ethopian kale (*B. carinata*) belong to the same botanical family Brassicaceae and the identical genus *Brassica*, they represent two different species showing dissimilar glucosinolate profiles. As the predominant glucosinolate, aliphatic alkenyl glucosinolate 2-propenyl was quantitatively determined in the leaves of *B. carinata*, whereas *B. oleracea* leaves were characterized by the indole 3-indolylmethyl as major glucosinolate (Fig. 2). The emphasis on 2-propenyl glucosinolate in the glucosinolate profile was also demonstrated by other *B. carinata* accessions (Schreiner, Krumbein, Mewis, Ulrichs, & Huyskens-Keil, 2009) suggesting the dominance of this alkenyl glucosinolate as specific characteristic of Ethiopian kale. Moreover, this distinct level of 2-propenyl glucosinolate enhances the potential of *B. carinata* as health promoter and chemo-preventive plant species (Lozano-Baena et al., 2015) as studies conducted on the biofunctional activities of 2-propenyl glucosinolate have revealed anti-cancer, anti-bacterial, anti-fungal, anti-oxidative, anti-inflammatory properties (Mazumder, Dwivedi, & du Plessis, 2016).

In contrast, the spiderplant *C. gynandra* is a member of the family Cleomaceae and mainly the aliphatic methyl glucosinolate (glucocapparin) occurred in their leaves (Fig. 2) showing consensus with previous investigations in which *C. gynandra* contained as most abundant glucosinolate also the methyl glucosinolate (Kjaer & Thomsen, 1963; Songsak & Lockwood, 2002). Generally the methyl glucosinolate and in addition the 2-hydroxy-2-methylbutyl glucosinolate (glucocleomin) appears to be the mostly present glucosinolates in other *Cleome* species, e.g. *C. chelidonii, C. viscosa* (Songsak & Lockwood, 2002) and in *C. serrulata* (Louda, Farris, & Blua, 1987), as well as *Cappara* species, e.g. caper (*Capparis sicula*) and bladderpod (*Isomeris arborea*) (Blua, Hanscom, & Collier, 1988; Conforti et al., 2011) belonging all to the overall order Brassicales. The health-promoting effects of methyl glucosinolate in *Cleome* has recently been studied suggesting to be partly effective against malaria, tuberculosis, pneumonia and fungal skin infection (Chinsembu, 2016).

Moreover, interestingly the three African species of the two families – Brassicaceae and Cleomaceae – considerably diverge in their indole glucosinolate composition. Spiderplant leaves showing just traces only of two indole glucosinolates: 3-indolylmethyl glucosinolate and 4-methoxy-3-indolylmethyl glucosinolate resulting in a strongly limited indole and overall glucosinolate composition. Compared to this focused

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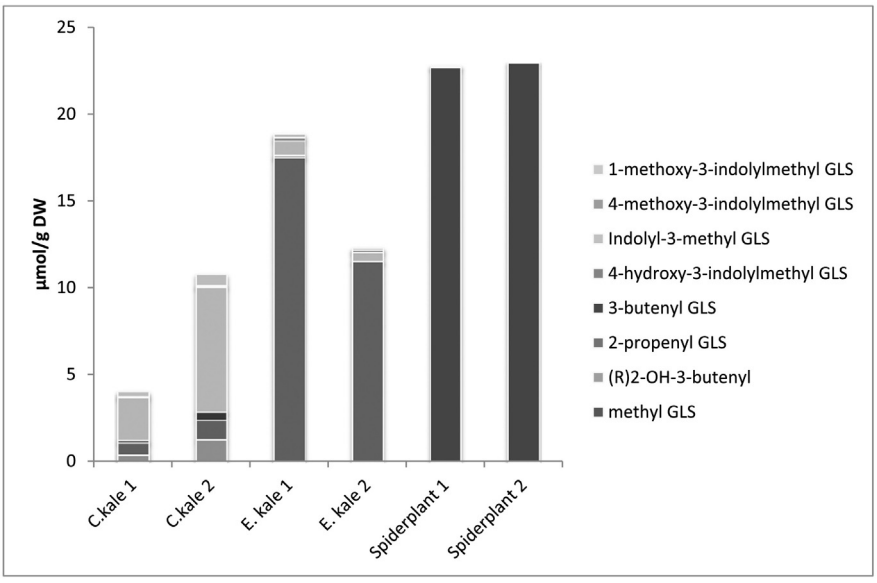

Fig. 3. Concentration of glucosinolates in indigenous African vegetables.

glucosinolate profile on nearly one individual glucosinolate (99% methyl glucosinolate of the total glucosinolate concentration) in spiderplant, in both *Brassica* species the indole glucosinolate composition comprises 3-indolylmethyl glucosinolate and its three derivatives, 4-hydroxy-3-indolylmethyl, 1-methoxy-3-indolylmethyl and 4-methoxy-3-indolylmethyl glucosinolates, even though partly in trace concentrations. Previous studies also revealed the complete absent of indole glucosinolates in *C. gynandra* (Songsak & Lockwood, 2002) which seems to be a unique characteristic of *Cleome* species. In contrast, common kale reveals a relative high indole 3-indolylmethyl glucosinolate level. As reported by other studies the concentration of 3-indolylmethyl glucosinolate in *B. oleracea* strongly depends on the variety and cultivar (e.g. reviewed by Verkerk et al., 2009). Hydrolysis products of 3-indolylmethyl glucosinolate are reported to be effective messenger against cancer development (Licznerska, Szaefer, Murias, Bartoszek, & Baer-Dubowska, 2016) and hence contributing to a healthy diet by ingestion of indigenous African leafy vegetables.

4. Conclusion

Indigenous African leafy vegetables vary enormously in their secondary plant metabolites. The genotype was shown to have a great impact on the secondary plant metabolites with respect to concentration and composition. Interestingly, quercetin glycosides can be found in high and medium concentrations in four species: namely African nightshade, spiderplant, amaranth, and cowpea. Additionally, the extraordinary hydroxycinnamic aicd glucaric isomers were tentatively identified in amaranth and spiderplant for the first time, and also firstly identified hydroxycinnamic acid isocitric isomers were present in high concentration in amaranth,. This is of interest for further structure-related-activity investigations focusing on the benefits of human nutrition for human health. In line with the health-relevant phenolic profile, amaranth and spiderplant include high concentrations of β-carotene, the pro-vitamin A. Moreover, the *Brassica* and *Cleome* species common kale, Ethiopian kale, and spiderplant also comprise glucosinolates of which only the ones in the *Brassica* species are well studied in relation to their anticanerogenic potential. Generally, a mixture of these indigenous African leafy vegetables can be recommended to contribute to different benefits such as antioxidant activity, increase pro-vitamin A or anticancerogenic compounds in a healthy diet.

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