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Profiling Glucosinolates and Phenolics in Vegetative and Reproductive Tissues of the Multi-Purpose Trees *Moringa oleifera* L. (Horseradish Tree) and *Moringa stenopetala* L.

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Moringa species are important multi-purpose tropical crops, as human foods and for medicine and oil production. There has been no previous comprehensive analysis of the secondary metabolites in *Moringa* species. Tissues of *M. oleifera* from a wide variety of sources and *M. stenopetala* from a single source were analyzed for glucosinolates and phenolics (flavonoids, anthocyanins, proanthocyanidins, and cinnamates). *M. oleifera* and *M. stenopetala* seeds only contained 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate at high concentrations. Roots of *M. oleifera* and *M. stenopetala* had high concentrations of both 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and benzyl glucosinolate. Leaves from both species contained 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and three monoacetyl isomers of this glucosinolate. Only 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate was detected in *M. oleifera* bark tissue. *M. oleifera* leaves contained quercetin-3-*O*-glucoside and quercetin-3-*O*-(6″-malonyl-glucoside), and lower amounts of kaempferol-3-*O*-glucoside and kaempferol-3-*O*-(6″-malonyl-glucoside). *M. oleifera* leaves also contained 3-caffeoylquinic acid and 5-caffeoylquinic acid. Leaves of *M. stenopetala* contained quercetin 3-*O*-rhamnoglucoside (rutin) and 5-caffeoylquinic acid. Neither proanthocyanidins nor anthocyanins were detected in any of the tissues of either species.

KEYWORDS: *Moringa oleifera*; glucosinolates; isothiocyanates; flavonoids; cinnamate esters; LC/MS

INTRODUCTION

Many species within the Capparales (glucosinolate-containing species) are known to have significant effects on human health (*1*, *2*). This is primarily due to the glucosinolates and the glucosinolate-derived hydrolysis products such as isothiocyanates and nitriles; generated upon cooking and consumption. The Moringaceae, within the Capparales, consists of a single genus (*Moringa*) divided into 10 species, and they are xerophytic species. On the basis of molecular analyses, this family is distantly related to the crucifers (Brassicaceae) with closer links to the Caricaceae (e.g., *Carica papaya*) (*3*). As well as *Moringa oleifera* (horseradish tree, drumstick) the Moringaceae includes the distinctive African bottle trees such as *M. ovalifolia* (phantom tree) and *M. stenopetala* (*4*). *M. oleifera* is used in a number of tropical countries as a human and animal crop, and also for medicinal purposes (*5*, *6*). The most widely reported use of *M. oleifera* is employment of the seeds for water purification and clarification (*7*, *8*). In addition, extracts of various *Moringa* tissues have been used as anti-cancer agents (*M. oleifera* seeds) (*9*), anti-trypanosomal agents (*M. stenopetala* leaves and roots) (*10*), and antiinflammatory and hepatoprotective agents (*M. oleifera* fruits and bark) (*11*, *12*). Incorporation of processed *M. oleifera* leaves into noodles improves nutritional quality by supplying the RDA for a number of vitamins (*13*). Leaf extracts have been shown to regulate thyroid status and cholesterol levels in rats (*14*, *15*). *M. oleifera* seed oil has also been investigated; in addition to having physical and chemical properties equivalent to olive oil, it is also high in tocopherols (*16*).

Several researchers have indirectly identified *Moringa* seed glucosinolates, using GC/MS of volatiles. These glucosinolates were isopropyl, isobutyl, 2-methylpropyl, 4-(α-L-rhamnopyranosyloxy)-benzyl and 4′-o-acetyl-4-(α-L-rhamnopyranosyloxy)-benzyl in *M. peregrina* and isopropyl, 2-methylpropyl, and traces of isobutyl and 4′-o-acetyl-4-(α-L-rhamnopyranosyloxy)-benzyl in *M. pterygosperma*, and 4-(α-L-rhamnopyranosyloxy)-benzyl in *M. oleifera* and *M. stenopetala* (*17*−*19*). Faizi et al. (*20*−*24*) have identified a number of biologically active components in *M.oleifera* clearly derived from glucosinolate hydrolysis prod-

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ucts i.e., isothiocyanates, nitriles, and derivatives of these compounds. A thiocarbamate (niaziminin) was isolated from leaf extracts and was shown to inhibit tumor-promoter-induced Epstein−Barr virus activation (*25*). Analysis of ethanol extracts of *M. stenopetala* leaves led to the identification of three major glycosides: rutin, and two derived from glucosinolates, namely 4-(4′*O*-acetyl-α-L-rhamnosyloxy)-isothiocyanate and 4-(4′*O*-acetyl-α-L-rhamnosyloxy)-benzaldehyde (*26*). A number of active principles isolated from *M. oleifera* leaves have been successfully synthesized, including novel rhamnose-substituted compounds (*27*, *28*).

Secondary metabolite data are often limited in many non-*Brassica* crops and this is the case for tissues of *Moringa* species, primarily because most analyses have been performed on *Brassica* and major European crops. It is important to characterize tissues that are consumed by humans, or are used in herbal medicines, because of potential health effects. This study undertook a comprehensive analysis of the major secondary metabolites in tissues of *M. oleifera* and *M. stenopetala*. Samples were analyzed using ion-pair LC/UV−Vis, LC/fluorescence detection (proanthocyanidins) and LC/ESI MS for the majority of secondary metabolites. The concentrations of *Moringa* secondary metabolites are considered both in relation to other Capparales and crop species and also their potential effects on human health.

MATERIALS AND METHODS

Plant Samples. Seeds of *M. oleifera* were obtained from one location in Nicaragua (Managua), five locations in Malawi (Bangula, Chikiwawa East Bank, Ngabu, Nsanje, Zion Church), one location in Tanzania (Pajaroyo) and one location in Senegal (Dakar). All were de-hulled, and seed kernels were dried at 100 °C for 24 h, before extraction and secondary metabolite analyses. Leaves and roots from 1 year-old trees in Nicaragua were harvested and freeze-dried. The leaf and root samples from Africa were initially air-dried and subsequently freeze-dried to ensure removal of residual water prior to milling and extraction. Some of the variation in secondary metabolite content reported for the African vegetative tissues in this study may be due to differences in drying temperature at the time of harvesting. The leaf samples represent the tissue used for human consumption and animal feed. Branches from 2 locations in Malawi were also analyzed: Bangula (1.8 cm diameter) and Nsanje (2.2 cm diameter). The bark was peeled from the branches, cut into 1 cm square pieces, flash frozen in liquid nitrogen and freeze-dried. Seeds of *Moringa oleifera* L. and *Moringa stenopetala* L. were also obtained from Dr. Martin L. Price (ECHO, N. Ft. Myers, FL). These seeds were sown in Shamrock potting compost and grown at 20−25 °C/18 °C day/night, 80−90% relative humidity with natural lighting between April and September. Samples were harvested when the young trees were 135 days old; samples were collected on dry ice and frozen at −20 °C before freeze-drying. All the vegetative samples, from all sources, were milled to a fine powder prior to extraction. All extractions were performed in triplicate in at least three separate experiments. *Moringa* voucher specimens are held at the University of Malawi and Proyecto Biomasa.

Chemicals. All chemicals were of analytical grade and were obtained from either Sigma or Aldrich. All solvents used in the extractions and analyses were of high-performance liquid chromatography (HPLC) grade, and all water was ultrapure (distilled, deionized, 18.2 MΩ). Kaempferol-3-*O*-glucoside (astragalin), quercetin-3-*O*-glucoside (isoquercitrin), quercetin 3-*O*-rhamnoside (quercitrin), and cyanidin 3-*O*-glucoside were obtained from Extrasynthese (France), quercetin 3-*O*-rhamnoglucoside (rutin) was obtained from Sigma. The 3-caffeoylquinic acid standard was obtained from Sigma and a partially purified sample of 5-caffeoylquinic acid was isolated from commercial UK plums (*29*). The kaempferol and quercetin malonylglucosides had previously been isolated from lettuce and endive species (*30*).

LC/MS Analysis. Seeds and other tissues were processed using methods previously detailed (*31*). All samples were analyzed in triplicate, using ion-pair liquid chromatography (LC) with either UV−vis (majority of metabolites) or fluorescence (proanthocyanidins) detection and also ion-pair LC/electrospray Ionization (ESI) mass spectrometer (MS) (to further confirm identities). The LC gradient for glucosinolate and phenolic analyses is a multi-purpose chromatographic method that simultaneously separates glucosinolates and phenolics (flavonoids, cinnamates, anthocyanins). Triplicate 40 mg samples were extracted in 1 mL 70% MeOH at 70 °C for 20 min and were then processed using the method previously detailed using sinigrin as the glucosinolate extraction standard (recovery was >98% for all samples) and quercetin 3-*O*-rhamnoside (quercitrin) as the phenolic extraction standard (recovery was >96% for all samples) (*31*). Samples were kept at 4 °C in the auto-sampler, and the injection volume was 20 μL. A photodiode array detector was used in combination with LC analyses: data was collected at 227 nm (glucosinolates and most UV-absorbing compounds), 270 nm (indoles and certain aromatic compounds), 325 nm (cinnamates), 370 nm (flavonoids), and 520 nm (anthocyanins), and overall between 200 and 600 nm. Glucosinolate and phenolic analyses were performed, using the negative ion electrospray ramped cone voltage method as previously described (*31*). Hydrolysis product analyses were performed using the same LC/MS system, Luna C_{18} (*2*) column and gradient conditions as described (*29*) but with the following MS modifications: capillary voltage = 3.4 kV; cone voltage = 27 eV; source block temperature = 120 °C; desolvation temperature = 350 °C; mass channel dwell time = 0.1 s; monitored at *m/z* 180.04, 202.04, 280.12, 302.10, 312.09, and 334.07. The analysis of proanthocyandin content in the tissues was achieved using the methods previously detailed (*32*). Triplicate 40 mg samples of freeze-dried tissue was extracted at 20 °C with 1 mL of 100% MeOH for 20 min, with vortex mixing every 5 min. The samples were centrifuged (17 000*g*, 4°C, 20 min) and filtered through a 0.2-μm PTFE filter (Chromos Express, Macclesfield, UK) prior to normal phase LC-fluorescence detection analysis. A sample of grape seed proanthocyanidins was used for comparison purposes. All sample analyses, for all of the metabolites, were performed in triplicate in at least three separate experiments.

RESULTS AND DISCUSSION

Glucosinolates were detected in all tissues of *M. oleifera* and *M. stenopetala*. The highest concentrations were found in the seeds (**Table 1**). Neither aliphatic nor indole glucosinolates were detected in any of the tissues analyzed. The MS data obtained for the glucosinolates gave the expected *m/z* values and fragment ions that were characteristic for these compounds: benzylglucosinolate, molecular weight of potassium salt 447; 408 (100%, $[M-H]^-$), 97 (92, $[SO_3H]^-$), 96 (32.7, $[SO_3]^-$); 4-(α-L-rhamnopyranosyloxy)-benxylglucosinolate, molecular weight of potassium salt 609; 570 (100%, $[M-H]^-$), 97 (59, $[SO_3H]^-$), 96 (12.4, $[SO_3]^-$); monoacetyl-4-(α-L-rhamnopyranosyloxy)-benxylglucosinolate isomers, molecular weight of potassium salt 651; isomer I: 612 (100%, $[M-H]^-$), 97 (75, $[SO_3H]^-$), 96 (15.5, $[SO_3]^-$); isomer II: 612 (100%, $[M-H]^-$), 97 (72.7, $[SO_3H]^-$), 96 (14.3, $[SO_3]^-$); isomer III: 612 (100%, $[M-H]^-$), 97 (72.0, $[SO_3H]^-$), 96 (13.7, $[SO_3]^-$). The seeds from Nicaragua and Africa only contained 4-(α-L-rhamnopyranosyl-oxy)-benzylglucosinolate (**Table 1, Figure 1**). 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate constituted a very high percentage of the seed dry weight (approximately 25%) in all the African samples.

A previous study (*33*) reported the general nutrient and antinutrient components in tissues of *M. oleifera*. These authors claimed that both cyanogenic glycosides and glucosinolates were present in trace amounts in twigs and stems, that they were undetectable in leaves, and that seeds contained 65.5 μmol glucosinolate/g seed (with a MW for the potassium salt of 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate of 609, which equates to approximately 40 mg/g or 4% of the seed weight). However, glucosinolate content was determined indirectly, using the

Table 1. Glucosinolates in Tissues from Mature *M. oleifera* Plants from Different Geographical Sources and from *M. oleifera* and *M. stenopetala* Plants Generated from ECHO Seed (glucosinolate content expressed as mean ± σ_{n-1} mg Compound /g dry weight of tissue)[a].

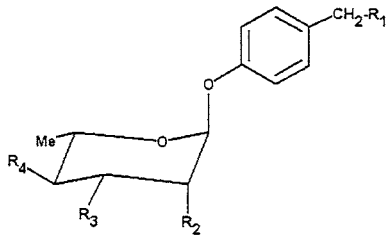
source	tissue	4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate	4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate monoacetyl-Isomer I	4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate monoacetyl-isomer II	4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate monoacetyl-isomer III	benzylglucosinolate
Bangula	seed	215 ± 52	0	0	0	0
Chikwawa	seed	264 ± 21	0	0	0	0
East Bank						
Ngabu	seed	227 ± 19	0	0	0	0
Nsanje	seed	250 ± 36	0	0	0	0
Pajaroyo	seed	220 ± 15	0	0	0	0
Zion Church	seed	252 ± 10	0	0	0	0
Dakar	seed	199 ± 25	0	0	0	0
Managua	seed	89 ± 4	0	0	0	0
Bangula	leaf	70.2 ± 4.1	1.8 ± 0.1	1.4 ± 0.4	4.9 ± 0.1	0
Nsanje	leaf	38.4 ± 0.6	0.8 ± 0.04	0.9 ± 0.07	1.2 ± 0.1	0
Zion Church	leaf	54 ± 2.4	2.3 ± 0.3	3.3 ± 0.3	32.4 ± 1.6	0
Dakar	leaf	47.2 ± 1.3	5.4 ± 0.3	2.8 ± 0.2	24 ± 1.3	0
Managua	leaf	48.3 ± 1.0	3.8 ± 0.07	1.8 ± 0.03	29.4 ± 0.7	0
Zion Church	young root	13 ± 2.2	0	0	0	6.8 ± 0.4
	old root (outer tissue)	2 ± 0.2	0	0	0	15.1 ± 0.9
	old root (inner tissue)	2.2 ± 0.1	0	0	0	0.6 ± 0.02
Managua	young root	5.4 ± 0.2	0	0	0	5.7 ± 0.03
Bangula	bark	22.1 ± 1.2	0	0	0	0
Nsanje	bark	10.2 ± 0.5	0	0	0	0
			plant material produced from ECHO seeds			
M. oleifera	seed	202 ± 15	0	0	0	0
M. stenopetala	seed	256 ± 11	0	0	0	0
M. oleifera	old leaves	33.9 ± 0.9	2.9 ± 0.06	1.2 ± 0.02	17.4 ± 0.4	0
M. stenopetala	old leaves	5.3 ± 0.5	0.6 ± 0.02	0.5 ± 0.1	5.1 ± 1.5	0
M. oleifera	young leaves	59.4 ± 1.4	5.0 ± 0.1	1.5 ± 0.08	50.2 ± 1.7	0
M. stenopetala	young leaves	8.2 ± 0.6	0.9 ± 0.04	0.5 ± 0.08	11.8 ± 1.5	0
M. oleifera	stem	16.3 ± 0.09	1.6 ± 0.01	0	3.2 ± 0.2	0
M. stenopetala	stem	13.6 ± 1.5	0	0	5.1 ± 2.7	30.5 ± 2.0
M. oleifera	whole root	20.4 ± 0.8	0	0	0	22.7 ± 0.7
M. stenopetala	whole root	40.9 ± 1.8	0	0	0	30.8 ± 1.7

[a] Trace = <0.01 mg compound/g dry weight of tissue.

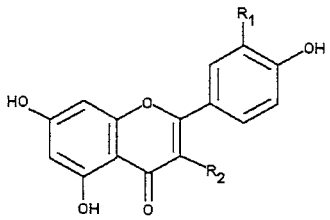
glucose-release assay that is known to be relatively insensitive (*34*). The presence of cyanide, and hence the assumption of cyanogenic compounds, can also be explained by the assay used, most likely detection of nitriles produced from the glucosinolates giving a false-positive result. The significantly lower amounts of 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate in the Managua seed was consistent; extraction of seeds from different generations contained similarly low amounts. Simple alkylglucosinolates have previously been reported in the seed of certain *Moringa* species (*17*−*19*). These were not detected in the *Moringa* species analyzed in this study; this was not due to poor extraction, because these types of glucosinolates were easily detected in seeds and other tissues of crucifers known to contain alkylglucosinolates (R. N. Bennett, unpublished data).

Leaf tissues from Nicaragua and Malawi contained four glucosinolates, two of which were present in significant concentrations (**Table 1, Figure 2**). These were identified as 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and three monoacetyl isomers of this glucosinolate. It would be necessary to purify the three monoacetyl-glucosinolates to determine the acetyl substitution positions by NMR. On the basis of the results of previous researchers, the major monoacetyl isomer is probably the 4″*O*-acetyl-4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate. The thiocarbamates and related compounds found by Faizi et al. (*20*−*24*) were not detected. It is likely that, following consumption of fresh leaves, both the isothiocyanates and nitrile from 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and its monoacetyl isomers are the major hydrolysis products and that thiocarbamates, etc., subsequently formed, are trace components. Treatment of a *M. oleifera* leaf glucosinolate extract (standard 40 mg extraction) with 3U *Sinapis alba* myrosinase produced a trace of 4-(α-L-rhamnopyranosyloxy)-benzylcyanide and four isothiocyanates (4-(α-L-rhamnopyranosyloxy)-benzylisothiocyanate, and three derived from the 4-monoacetyl-4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate isomers in the same ratios as the parent glucosinolates) (**Figure 2**). Roots of *M. oleifera* contained only benzylglucosinolate and 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate, and the bark tissues contained only 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate (**Table 1**). The 4-monoacetyl-4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate isomers appear to be restricted to the leaves of *M. oleifera*. *M. stenopetala* leaf tissues contained lower amounts of the 4-monoacetyl-4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate isomers but had significant amounts of 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate in stem tissue (**Table 1**). Root tissues from all the samples of both species contained both 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and benzylglucosinolate (**Table 1**). The glucosinolates are restricted to the plant order Capparales; they are not found in any other plants. Within the Capparales, there is a

Isopropylglucosinolate	$(CH_3)_2$-CH_2-CH(=$NOSO_3^-K^+$)-S-Glc
2-Methylpropylglucosinolate	$(CH_3)_2$-CH-CH_2-CH(=$NOSO_3^-K^+$)-S-Glc
Isobutylglucosinolate	$(CH_3)_3$-C-CH(=$NOSO_3^-K^+$)-S-Glc



	R1	R2	R3	R4
4-(α-L-Rhamnopyranosyloxy)-benzylglucosinolate	-CH(=$NOSO_3^-K^+$)-S-Glc	OH	OH	OH
4"-Acetyl-4-(α-L-Rhamnopyranosyloxy)-benzylglucosinolate	-CH(=$NOSO_3^-K^+$)-S-Glc	OH	OH	O-Ac
4-(α-L-Rhamnopyranosyloxy)-benzylisothiocyanate	-NCS	OH	OH	OH
4-(α-L-Rhamnopyranosyloxy)-benzylcyanide	-CN	OH	OH	OH



	R1	R2
Kaempferol 3-*O*-glucoside	H	Glc
Kaempferol 3-*O*-(6"-malonylglucoside)	H	Glc-6"-O-CO-CH_2-COOH
Quercetin 3-*O*-glucoside	OH	Glc
Quercetin 3-*O*-(6"-malonylglucoside)	OH	Glc-6"-O-CO-CH_2-COOH
Quercetin 3-*O*-rhamnosylglucoside (Rutin)	OH	Glc-Rham

Figure 1. Structures of glucosinolates previously reported in *Moringa* species and flavonoids identified in this investigation.

wide distribution of aromatic and hydroxy-aromatic glucosinolates. However, the distribution of sugar-modified aromatic glucosinolates is much more restricted. So far, these sugar-modified glucosinolates have been found in *Moringa* species (Moringaceae: 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate and monoacetyl derivatives), *Reseda* species (Resedaceae, 2-(α-L-rhamnopyranosyloxy)-benzylglucosinolate) and *Hesperis* species (Brassicaceae, 4-(α-L-apiosyloxy)-benzylglucosinolate, 3-hydroxy, 4-(α-L-apiosyloxy)-benzylglucosinolate and derivatives) (*17*−*19*, *35*, *36*).

The seeds of *M. oleifera* and *M. stenopetala* did not contain any detectable levels of cinnamic acid choline esters such as sinapine, as found in other crucifer species (*36*), nor were choline esters detected in other tissues. However, leaves of *M. oleifera* contained appreciable concentrations of two cinnamate esters. These were identified by LC/MS, and by comparison with standards, as 3-caffeoylquinic and 5-caffeoylquinic acid (**Table 2**, **Figure 2**). Only 5-caffeoylquinic acid was found in leaves of *M. stenopetala* (**Table 2**). The MS data obtained for the phenolics gave the expected *m*/*z* values and fragment ions that were characteristic for these compounds: 3-caffeoylquinic acid, molecular weight 354; 353 (80%, [M−H]-), other characteristic ions (191 [100], 179 [69], 135 [26]); 5-caffeoylquinic acid, molecular weight 354; 353 (100%, [M−H]-), other characteristic ions (179 [74.6], 173 [92.6], 135 [17.9]). Chlorogenic acids (3-caffeoylquinic, 5-caffeoylquinic and other mono- and di-cinnamoylquinic acids) are found in many plant species, including members of the Capparales (*37*, *38*). Previous analyses of crucifers have shown that, when present, 3-caffeoylquinic acid is usually predominant, although other cinnamoyl quinic acids have been reported (*38*). The amounts vary, depending on tissue and species (e.g., leafy *Brassica* species 6−120 mg/kg fresh weight)(*38*). The amounts in *Moringa* leaves varied, depending on the location, but in all samples, the concentration of 5-caffeoylquinic was higher than 3-caffeoylquinic, except for the tissues from the *M. oleifera* trees produced

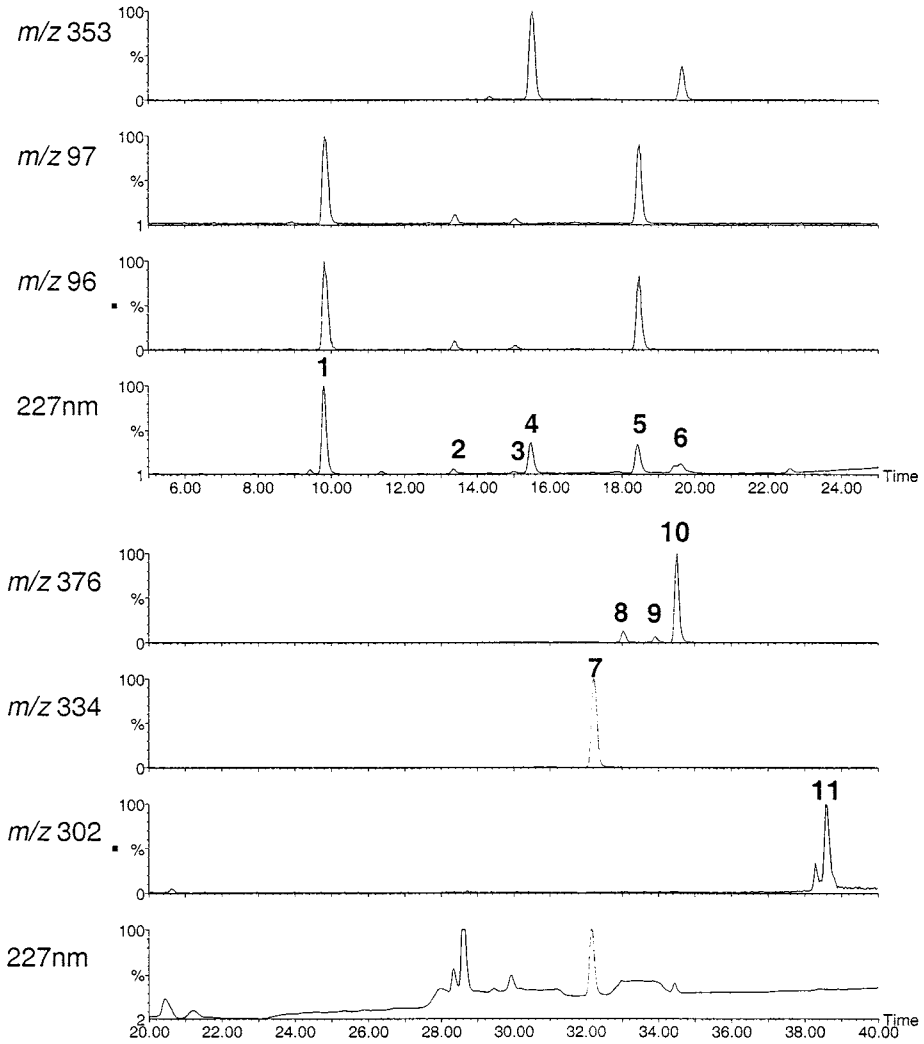


Figure 2. LC/ES− MS data for leaves of *M. oleifera* with *m*/*z* 353 ([M−H]$^-$ for 5-caffeoylquinic and 3-caffeoylquinic acid), *m*/*z* 96/97 (glucosinolate-specific ions) and UV trace (227 nm). Peak ID: 1 = 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate, 2 = Monoacetyl-isomer I (of peak 1), 3 = Monoacetyl-isomer II (of peak 1), 4 = 5-caffeoylquinic (neochlorogenic acid), 5 = Monoacetyl-isomer III (of peak 1), and 6 = 3-caffeoylquinic acid (chlorogenic acid). LC/ES+ MS data for glucosinolate hydrolysis products derived from treatment of a leaf extract with exogenous *S. alba* myrosinase. Peak ID: 7 = 4-(α-L-rhamnopyranosyloxy)-benzylisothiocyanate ([M+Na]$^+$ = 334), 8/9/10 = 4-Monoacetyl-4-(α-L-rhamnopyranosyloxy)-benzylisothiocyanate isomers [M+Na]$^+$ = 376), 11 = 4-(α-L-rhamnopyranosyloxy)-benzylcyanide ([M+Na]$^+$ = 302).

from ECHO seed (**Table 2**). Three major and two minor flavonoids were detected in leaves of *M. oleifera* and were identified as quercetin 3-*O*-glucoside, quercetin 3-*O*-(6″-malonylglucoside), an unidentified minor quercetin 3-*O*-malonylglucoside isomer, kaempferol 3-*O*-glucoside, and kaempferol 3-*O*-(6″-malonylglucoside) (**Table 2, Figure 3**). The MS data obtained for the flavonoids gave the expected *m*/*z* values and fragment ions that were characteristic for these compounds, including the loss of CO_2 from the malonylated flavonoids: kaempferol 3-*O*-glucoside, molecular weight 448; 447 (100%, [M−H]$^-$), 285 (32.2, [Aglycone-H]$^-$); kaempferol 3-*O*-(6″-malonylglucoside), molecular weight 534; 533 (39.1%, [M−H]$^-$), 489 (100, [(M−H)-CO_2]-), 285 (9.0, [Aglycone-H]$^-$); quercetin 3-*O*-glucoside, molecular weight 464; 463 (100%, [M−H]$^-$), 301 (3.7, [Aglycone-H]$^-$); quercetin 3-*O*-(6″-malonylglucoside), molecular weight 550; 549 (42.9%, [M−H]$^-$), 505 (100, [(M−H)-CO_2]-), 301 (4.3, [Aglycone-H]$^-$); quercetin 3-*O*-(X″-malonylglucoside), molecular weight 550; 549 (32.9%, [M−H]$^-$), 505 (100, [(M−H)-CO_2]-); quercetin 3-*O*-rhamnosylglucoside, molecular weight 610; 609 (89%, [M−H]$^-$), 463 (100, [(M-Rhamnose)-H]$^-$), 301 (14.0, [Aglycone-H]$^-$). Leaves of *M. oleifera* and *M. stenopetala* produced in the UK had much lower flavonoid levels, possibly due to lower light intensity. Leaves of *M. stenopetala* contained quercetin 3-*O*-rhamnosylglucoside (rutin) and traces of quercetin 3-*O*-glucoside but none of the kaempferol or malonylated glucosides (**Table 2**). Low levels of flavonoids were present in the stem tissues of young trees but not in the older trees. The distribution of flavonols (that include kaempferol, quercetin, isorhamentin, rhamnetin, etc.) is broad within the plant kingdom (*39*). Kaempferol 3-*O*-glucoside (astragalin) has been found in many plants, but kaempferol 3-*O*-(6″-malonylglucoside) and related malonylglucosides are more restricted, e.g., chickpea leaves, endive leaves, petunia flowers (with additional hydroxy-cinnamoyl groups), *Haplophyllum pedicellatum*, *Gerbera* flowers, and *Clitorea ternatea* flowers (*30*, *40*−*43*). Similar distribution patterns are found for quercetin 3-*O*-glucoside (isoquercitrin) and quercetin 3-*O*-rhamnosylglucoside (rutin), but quercetin 3-*O*-(6″-malonylglucoside) is more restricted, e.g., lettuce, aquatic *Ranuculus* species, *Gerbera* flowers, and *Clitorea ternatea* flowers (*30*, *43*−*45*). In general, malonylation and acylation of flavonoids appears to be generally more common

Table 2. Flavonoids and Chlorogenic Acids in Old Leaves, Young Leaves, and Stem Tissues of Young *M. oleifera* and *M. stenopetala* Trees and in Young Leaves of Mature *M. oleifera* Trees (mean ± σ_{n-1} mg compound g^{-1} DW of Tissue)[a].

		compound							
species, age, sample source	tissue	3-caffeoyl-quinic acid	5-caffeoyl-quinic acid	kaempferol 3-*O*-Glc	kaempferol 3-*O*-(6″-malonyl-Glc)	qercetin 3-*O*-Glc	quercetin 3-*O*-(6″-malonyl-Glc)	quercetin 3-*O*-(X″-malonyl-Glc)	quercetin 3-*O*-Glc-rham
					M. oleifera				
young (ECHO)	old leaves	6.2 ± 0.2	2.0 ± 0.06	0.16 ± 0.01	0.14 ± 0.01	0.31 ± 0.02	0.27 ± 0.01	nd	nd[b]
	young leaves	8.9 ± 0.3	1.1 ± 0.05	0.08 ± 0.01	0.08 ± 0.02	0.21 ± 0.01	0.25 ± 0.02	trace[c]	nd
	whole stem	0.3 ± 0.01	nd	0.14 ± 0.01	Trace	0.08 ± 0.01	0.04 ± 0.01	nd	nd
old, Bangula	young leaves	0.7 ± 0.05	1.4 ± 0.09	0.9 ± 0.07	0.3 ± 0.02	2.9 ± 0.2	3.4 ± 0.2	0.2 ± 0.02	nd
old, Nsanje	young leaves	0.38 ± 0.02	0.52 ± 0.01	1.8 ± 0.02	1.0 ± 0.05	2.2 ± 0.04	0.9 ± 0.02	trace	nd
old, Zion Church	young leaves	0.6 ± 0.05	1.3 ± 0.06	1.6 ± 0.08	3.1 ± 0.09	2.6 ± 0.1	3.9 ± 0.1	0.3 ± 0.02	nd
old, Dakar	young leaves	1.3 ± 0.08	1.9 ± 0.1	1.4 ± 0.01	0.9 ± 0.01	6.3 ± 0.3	3.5 ± 0.2	0.1 ± 0.01	nd
old, Managua	young leaves	1.9 ± 0.01	4.1 ± 0.08	0.9 ± 0.01	2.6 ± 0.05	4.1 ± 0.2	10.8 ± 0.4	0.3 ± 0.01	nd
					M. stenopetala				
young (ECHO)	old leaves	nd	4.2 ± 0.4	nd	nd	nd	nd	nd	2.6 ± 0.1
	young leaves	nd	6.2 ± 0.3	nd	nd	nd	nd	nd	3.0 ± 0.1
	whole stem	nd	nd	nd	nd	0.19 ± 0.08	nd	nd	0.16 ± 0.06

[a] Trace = <0.01 mg compound g^{-1} DW of tissue. [b] nd = not detected. [c] Neither roots of young trees nor roots, bark, and seeds of the old trees contained detectable levels of flavonoids or chlorogenic acids.

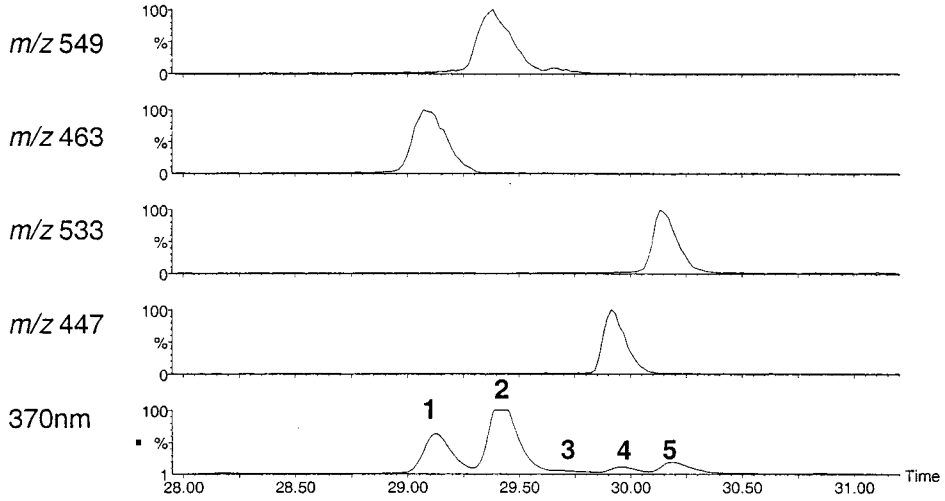


Figure 3. LC/MS and UV trace (370 nm) of flavonoids in leaves Peak ID: 1 = quercetin 3-*O*-Glc (*m*/*z* = 463), 2 = quercetin 3-*O*-(6″-malonyl-Glc) (*m*/*z* 549), 3 = quercetin 3-*O*-(X″-malonyl-Glc) (*m*/*z* 549), 4 = kaempferol 3-*O*-Glc (*m*/*z* 447), 5 = kaempferol 3-*O*-(6″-malonyl-Glc) (*m*/*z* 533)

in floral tissues and with the anthocyanin glycosides, rather than the flavonol glycosides (*46*). Acylated flavonoids have been found in Capparales species such *Arabidopsis thaliana*, radish (*Raphanus sativus*), and red cabbage (*Brassica oleracea*) (*47*−*49*). Analysis of tissues of *Moringa* species for the presence of proanthocyanidins has not been previously performed. On the basis of retention times relative to a previously characterized grape seed extract, the specific excitation and emission wavelengths, and MS analyses, no proanthocyanidins were detected in any *Moringa* tissues. The bark and root tissues of *M. oleifera* and *M. stenopetala* did contain low levels of tannin-like substances but these were not characterized.

M. oleifera tissues are used principally as foods (leaves, flower buds, roasted seed kernels), herbal medicines (all tissues), animal feed (leaves, stem, and seed meal processed to remove the coagulating proteins), a source of tannins and dyes (bark), for fuel (seed husks), and as a male aphrodisiac (seed kernels). *Moringa* tissues have been consumed in Africa, India, and Nicaragua for many years. Therefore, the secondary metabolites in the tissues consumed by humans and animals have the potential to affect health. There are considerable data on the effects of glucosinolates, and more specifically, their hydrolysis products, and there are increasing data on the absorption, disposition, metabolism and excretion of dietary isothiocyanates from *Brassica* and common crop species (*50*, *51*). There are no comparable data for either the rhamnose-substituted glucosinolates or their hydrolysis products generated upon consumption of *Moringa* tissues. The many papers of Faizi et al. (*20*−*24*) have provided data on the presence of various byproducts of the isothiocyanates and nitriles clearly derived from the *Moringa* glucosinolates. However, these appear to be minor components in comparison with the concentrations of isothiocyanates and nitriles that have the potential to be released upon consumption of *Moringa* tissues. The leaves of *M. oleifera* are unusual, compared with other Capparales species, because they contain significant levels not only of glucosinolates but also of flavonoids and cinnamate esters. Considerable information on the absorption, disposition, metabolism and excretion, and in vitro and in vivo effects of flavonoids is available (*52*−*54*). Cinnamate esters may constitute a significant part of the total phenolics in the human diet, especially in heavy coffee drinkers (*55*). It has previously been shown that 3-caffeoylquinic acid can be absorbed in the human small intestine and that cinnamoylquinic acids can be metabolized in the colon of

humans by the microbial flora (*56*, *57*). Complete knowledge of the secondary metabolite content of human foods and potential new crops is essential, so that an evaluation can be made on both the potential health related effects; both positive (e.g., anti-carcinogenic) and negative (e.g., toxicity due to the presence of anti-nutritional factors or natural toxicants and adverse effects on drug absorption and metabolism).

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