Ontogenic Profiling of Glucosinolates, Flavonoids, and Other Secondary Metabolites in *Eruca sativa* (Salad Rocket), *Diplotaxis erucoides* (Wall Rocket), *Diplotaxis tenuifolia* (Wild Rocket), and *Bunias orientalis* (Turkish Rocket)

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As an influence of the Mediterranean diet, rocket species such as *Eruca sativa* L., *Diplotaxis* species, and *Bunias orientalis* L. are eaten all over the world at different ontogenic stages in salads and soups. They are all species within the plant order Capparales (glucosinolate-containing species), and all are from the family Brassicaceae. Predominantly, the leaves of these species are eaten raw or cooked, although *Eruca* flowers are also consumed. There is considerable potential with raw plant material for a higher exposure to bioactive phytochemicals such as glucosinolates, their hydrolysis products, and also phenolics, flavonoids, and vitamins such as vitamin C. These compounds are susceptible to ontogenic variation, and the few published studies that have addressed this topic have been inconsistent. Thus, an ontogenic study was performed and all samples were analyzed using a previously developed robust liquid chromatography/mass spectrometry method for the identification and quantification of the major phytochemicals in all tissues of the rocket species. Seeds and roots of both *Eruca* and *Diplotaxis* contained predominantly 4-methylthiobutylglucosinolate. Leaves of *Eruca* and *Diplotaxis* contained high amounts of 4-mercaptobutylglucosinolate with lower levels of 4-methylthiobutlyglucosinolate and 4-methylsulfinylbutylglucosinolate. Flowers of *Eruca* and *Diplotaxis* contained predominantly 4-methylsulfinylbutyl-glucosinolate. In addition, roots of both *Diplotaxis* species contained 4-hydroxybenzylglucosinolate but 4-hydroxybenzylglucosinolate was absent from roots of *Eruca*. Seeds and seedlings of all *Eruca* contained *N*-heterocyclic compounds but no sinapine, whereas *Diplotaxis* contained sinapine but not the *N*-heterocycles. In all tissues of *B. orientalis*, 4-hydroxybenzylglucosinolate and 4-methylsulfinyl-3-butenylglucosinolate were predominant. All rocket tissues, except roots, contained significant levels of polyglycosylated flavonoids, with/without hydroxycinnamoyl acylation. The core aglycones were kaempferol, quercetin, and isorhamnetin. The exception was *B. orientalis*, which had a negligible seed flavonoid content as compared with the other species. Anthocyanins were only detected in *Eruca* flowers and consisted of a complex pattern of at least 16 different anthocyanins.

KEYWORDS: *Eruca sativa*; *Diplotaxis tenuifolia*; *Diplotaxis erucoides*; *Bunias orientalis*; salad crucifers; glucosinolates; complex flavonoids; sinapine; anthocyanins, ontogenic regulation; LC/MS

INTRODUCTION

The Capparales, glucosinolate-containing plants, and specifically those within the family Brassicaceae play a major role in worldwide vegetable production and consumption, ranking second after the Solanaceae, e.g., potatoes and tomatoes. Brassicaceae is a large family of plants including major veg-

etable crops such as broccoli and cabbage and also salad and herb species such as *Eruca sativa* L. (also known as rocket or rucola), *Diplotaxis erucoides* L. (wall rocket), *Diplotaxis tenuifolia* L. (wild or sand rocket), and *Bunias orientalis* L. (Turkish rocket; Turkish warty cabbage) (*1*). These species, well-represented in the Mediterranean area, have gradually spread to other latitudes, and there has been increasing interest in the past decade for their use in salads (simple or in mixtures), although cooked leaves are also frequently used and also more recently sprouted seedlings. Leaves of salad rocket (*E. sativa*

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L.) are a popular salad crop eaten by humans, flowers of *Eruca* are also used in salads, and the seedlings may have potential as a functional food. Although roots of these species are not currently consumed, rocket species are readily grown hydroponically; thus, easy harvesting of root tissue is also possible. Leaves are characterized by a pungent taste dependent on species, genetic diversity, and environment (*2*). Rocket has been reported as having a high vitamin C content and also a medicinal plant with the following properties: antiphlogistic, astringent, diuretic, an aid to digestion, stimulant, laxative, antacid, antiinflammatory for colitis, and also effects on blood circulation (*3*, *4*). Eating the fresh raw material is probably the best way of getting all of the benefits claimed for this vegetable since only minor losses in beneficial components are likely to occur, primarily during storage. In India and Pakistan, special ecotypes are cultivated for their inedible and pungent oil seed production (*5*). In ancient times, *Eruca* spp. were also used as biological controls to inhibit pest development (*6*).

Limited previous data have shown that rocket species contain glucosinolates; the data are predominantly for seed glucosinolates. About 120 glucosinolates have been identified in different Capparales species and can be classified into three basic categories: aliphatic, aromatic, and indole according to the type of side chain (R). Glucosinolates coexist in the plant with their degradative enzyme thioglucoside glucohydrolase (E.C. 3.2.1.147), also known as myrosinase. Glucosinolates are sequestered in the plant vacuoles, and myrosinase is localized in the cytosol and is also present at high levels in specialized cells called myrosin cells (*7*). During consumption, the glucosinolates and myrosinase combine leading to the formation of glucose and an unstable intermediate that degrades to produce a sulfate ion and a variety of bioactive products including isothiocyanates, thiocyanates, and nitriles. The hydrolysis products formed are dependent on the glucosinolate structure and reaction conditions, e.g., pH or the presence of Fe^{2+} or epithiospecifier protein. Unless a crucifer is a major crop, such as the *Brassica* species, then phytochemical data are often incomplete for the whole plant, and often, the focus has been on the glucosinolate content alone (*8*−*10*). Tissues, other than those consumed by animals or humans, are rarely analyzed, hence, the lack of root and floral phytochemical data for many species.

Seeds of *E. sativa* have been reported to contain mainly 4-methylthiobutyl-glucosinolate and a small amount of 4-methylsulfinylbutylglucosinolate (4-MSB) (*8*, *11*). A structurally unique isothiocyanate was identified in *E. sativa* leaves (*12*). Recently, it has been shown that the major glucosinolate in leaves of *E. sativa* L. is 4-mercaptobutylglucosinolate (4-MER), probably derived from *S*-demethylation of 4-methylthiobutylglucosinolate (4-MTB) (*13*). This glucosinolate upon hydrolysis produces 4-mercaptobutylisothiocyanate—a volatile and very pungent compound that may in part explain the distinct odor of salad rocket (*13*). Seeds of *Diplotaxis* species and *B. orientalis* have previously been reported to contain 4-MTB and 4-MSB (*8*).

In the past two decades, there has been increasing interest in the bioavailability and biological effects of phenolics and flavonoids in food plants. They are known to have direct antioxidant and free radical-scavenging activities but can also induce expression of various genes encoding metabolic enzymes thought to decrease the risk of various diseases and disorders, e.g., cancer, coronary heart disease, and immune dysfunctions (*14*−*18*). There have been far more analyses of phenolics and flavonoids in the Brassicaceae, and specifically in *Brassica* food plants, than in any other Capparales species. There are limited
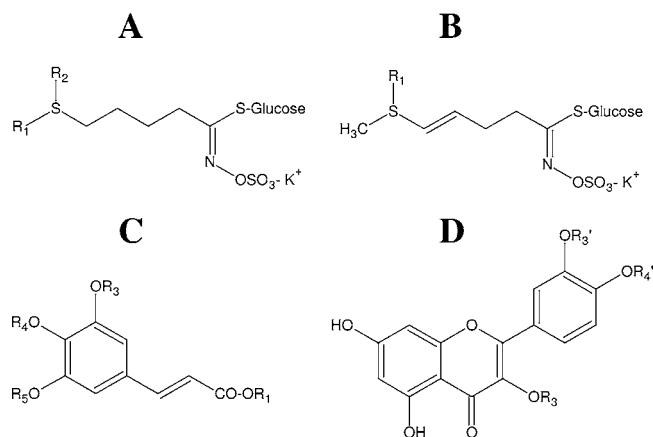
data on the phenolic and flavonoid content of rocket species: qualitative analyses by liquid chromatography/mass spectrometry (LC/MS) and NMR of quercetin triglucosides in *E. sativa* (*19*), initial qualitative thin-layer chromatography identification of various kaempferol, quercetin, and isorhamnetin glycosides in leaves of flowering *Diplotaxis* species (*20*), and initial semiquantitative LC-diode array detection identification of flavonoids in leaves of nonflowering *Eruca vesicaria* and *D. tenuifolia* (*21*). Recently, an analysis of flavonoids in Brazilian foods, including *E. sativa* leaves, showed that the leaves contained 410−1180 µg/g fresh weight total kaempferol; the kaempferol glycosides were not further characterized (*22*). In leaves of *B. orientalis*, various hydroxy-cinnamate derivatives, including sinapoylglucose, and ionone-derived terpenes were found; no data on glucosinolates or flavonoids were reported (*23*).

During the last two decades, consumption of fruits and vegetables has received increased recommendations based on epidemiological studies (*24*−*27*). Within the vegetables, *Brassica* are of high nutritional and health-promoting value (*28*). Glucosinolates, and more specifically their hydrolysis products, have also been reported to take part in plant−environmental interactions, e.g., antimicrobial and allelopathic activities (*9*). Among their known effects in humans, they have been shown to modulate the activity of phase I (inhibited) and phase II (induced) drug-metabolizing enzymes and also protein kinases involved in signal pathways (*29*−*33*). Medical research on crucifers has increased because of the potential beneficial effects to human health of the glucosinolate-derived hydrolysis products such as isothiocyanates and indoles; that is, they may reduce the risk of gastrointestinal and breast cancers (*29*, *34*). Among the glucosinolate hydrolysis products from *Brassica*, sulforaphane, the isothiocyanate derived from 4-MSB (aka glucoraphanin), is probably one of the most well-known inducers of anticarcinogenic processes (*34*).

There is increased interest in using crucifers and related Capparales as functional foods, e.g., broccoli sprouts (*35*). There are also considerable possibilities in using raw leafy salad species to deliver higher concentrations of health-promoting bioactives. Therefore, this gap in the phytochemical knowledge of these food species led us to complete an extensive and detailed study on secondary metabolites in tissues of *Eruca*, *Diplotaxis*, and *B. orientalis* since this plant material can be used at different ontogenic stages.
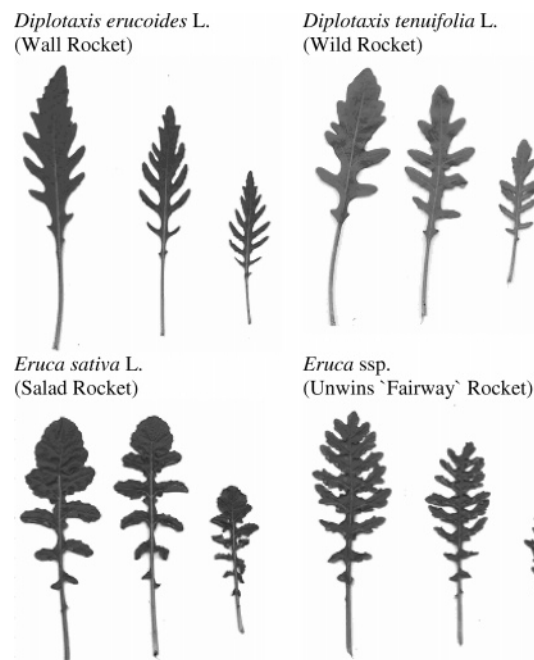
MATERIALS AND METHODS

Plant Material. All seeds were obtained from commercial sources: *E. sativa* L. [salad rocket; B & T World Seeds, Paguignan, Olonzac, France; Future Foods, Luckleigh Cottage, Hockworthy, Wellington, Somerset, United Kingdom; E. W. Kings, United Kingdom; Moles Seeds, Colchester, Essex, United Kingdom (standard and skyrocket); Nickersons, United Kingdom; Seeds of Italy, London, United Kingdom], *D. erucoides* L. [wall rocket; Nickersons; Seeds of Italy (standard and special types)], *D. tenuifolia* L. [wild rocket; B & T World Seeds; The Herbary, Horningham, Wiltshire, United Kingdom (olive-leafed rocket)], Fairway rocket (Unwins Seeds Ltd., Cambridge; no detail available but probably *Eruca* ssp. from seed size, color, and shape), and *B. orientalis* L. (Turkish rocket; B & T World Seeds). For the seedling time−course studies, seeds were sown on filter paper, grown in a propagator box with a day/night temperature of 20−25/18 °C under natural lighting between May and August with supplementary lighting (12 h day, combination of tungsten and fluorescent lamps, 350 µE/m/s average PAR), and 80/90% day/night relative humidity. Samples were collected at 3, 5, and 7 days postwetting. For mature plant material, seeds were sown in 12 cm diameter pots (approximately 0.5 L volume) containing John Innes no. 1 plant compost (12 seeds per pot, thinned down to one plant per pot 1−2 weeks later), and grown at 20−25/18

A

R₂—S—(chain)—S-Glucose
R₁
N—OSO₃⁻ K⁺

B

R₁—S—(chain)—S-Glucose
H₃C
N—OSO₃⁻ K⁺

C

OR₃
R₄O—(ring)
R₅O—(chain)—CO-OR₁

D

OR₃'
OR₄'
HO—(flavonoid ring)—OR₃
OH
O

Figure 1. Structures of phytochemicals previously detected in *Eruca*, *Diplotaxis*, and *Bunias* rocket species. Structures: (**A**) 4-MTB (R1 = CH₃, R2 = no substitution), 4-MER (R1 = H, R2 = no substitution), and 4-MSB (R1 = CH₃, R2 = O=); (**B**) 4-methylthio-3-butenylglucosinolate (R1 = no substitution) and 4-MS3B (R1 = O=); (**C**) ferulic acid (R1 = R5 = H, R3 = OCH₃, and R4 = OH), sinapic acid (R1 = H, R3 = R5 = OCH₃, and R4 = OH), and sinapoyl-glucose (R1 = C₆H₁₁O₅ [Glc], R3 = R5 = OCH₃, and R4 = OH); and (**D**) core flavonoid structures, kaempferol (R3' = H, R3 = R4' = OH), quercetin (R3' = R3 = R4' = OH), and isorhamnetin (R3' = OCH₃, R3 = R4' = OH).

Figure 2. Leaf morphology of the different *Eruca* and *Diplotaxis* species investigated.

°C day/night under the same conditions as for the seedlings. Ages of plants for specific tissues are detailed in the data tables.

Chemicals. All chemicals were of analytical grade and were obtained from Sigma/Aldrich. All solvents were of high-performance liquid chromatography (HPLC) grade, and all water was ultrapure. All flavonoid glycoside standards had either been previously purified from broccoli (kaempferol and quercetin 3-*O*-sophorosides) (*36*) or were obtained from a commercial source (aglycones, mono-3-*O*-glucosides, and 3-*O*-rutinosides of kaempferol, quercetin, and isorhamnetin from Extrasynthese, Genay, France). The reducing agent tris(2-carboxyethyl) phosphine, used to prevent formation of 4-MER disulfide artifacts, was obtained from Fluka (United Kingdom). Indole-3-acetonitrile was obtained from Sigma-Aldrich. Intact glucosinolate standards and sinapine had been previously purified using a combination of Ecteola Cellulose solid phase extraction (glucosinolates) and polyamide solid phase extraction (flavonoids) and preparative HPLC

(*13*, *36*). For all standards, HPLC calibration curves were constructed by injection of 20 μL of different stock concentrations of the standards; the calibrations for phenolics and glucosinolates were linear up to 2 mg/mL.

Extraction and Analyses of Phytochemicals. For the seedling studies, tissue harvested each day was dried at 100 °C for 16 h. This was done because in the early stages a considerable proportion of the material was seed and previous studies have shown that this drying process effectively inactivates the high myrosinase activity in seed but does not cause secondary metabolite degradation (see the Supporting Information; in addition, control foliar tissue treated this way, as compared with freeze drying, showed <0.1% difference in glucosinolates, flavonoids, choline esters, etc.). Tissues from the adult plants (roots, young leaves, and flowers) were flash frozen in liquid N₂ and freeze-dried for 7 days. After they were dried, the samples were milled to a fine powder. Replicate samples (3 × 40 mg) were extracted with

Table 1. Glucosinolates, Phenolics, and *N*-Heterocyclic Compounds in Seeds of Commercial *E. sativa*, *D. erucoides*, *D. tenuifolia*, and *B. orientalis* from Different Sources (Each Compound Expressed as mg/g Fresh Weight Seed)[a]

species	source (type of seed and cultivar if listed)	glucosinolates				phenolics	*N*-heterocylces[b]				
		4-MTB	4-MSB	4-MS3B	4-HB	sinapine	N1	N2	N3	N4	N5
E. sativa	B & T World Seeds (Victoria)	E	A				A	A	A	A	A
	Future Foods (standard)	E	A				A	A	A	A	A
	E. W. Kings (standard)	F	A				A	A	A	A	A
	Moles (standard)	E	A				A	A	A	A	A
	Moles (Skyrocket)	F	A				A	A	A	A	A
	Nickersons (standard)	E	A				A	A	A	A	A
	Seeds of Italy (standard)	E	A				A	A	A	A	A
D. erucoides	Nickersons (standard)	D	A			A					
	Seeds of Italy (standard)	D	A			A					
	Seeds of Italy (special quality)	D	A			A					
D. tenuifolia	B & T World Seeds (standard)	F	A			A					
	The Herbary (olive-leafed)	D	A			A					
Eruca ssp. (Fairway)	Unwins (details unavailable)	E	A				A	A	A	A	A
B. orientalis	B & T World Seeds (standard)		A	B	D	A					

[a] Intact glucosinolates are expressed as K⁺ salts; *N*-heterocycles are expressed as indole-3-acetonitrile equivalents. Concentrations are shown as letter codes: blank cell, not detected; A, 1–10; B, 10–20; C, 20–30; D, 30–40; E, 40–50; and F, 50–60. Please note that the letter codes in each table, representing compound concentrations, are different depending on the tissue; for example, the seed content is much higher than other tissues and thus the letters representing glucosinolate ranges are in milligrams as compared with other tissues where microgram ranges are used, e.g., in vegetative and floral tissue tables. [b] Although individual concentrations of the *N*-heterocycles were consistently <10 mg/g FW, there were differences in the ratios (N1:N2:N3:N4:N5 = 0.05:0.30:0.18:1.00:0.26).

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1.5 (for all seed and seedling samples) or 1.0 mL (for all other samples) of 70% v/v methanol. To one replicate of each sample, both 50 μL of 12 mM sinigrin (glucosinolate extraction standard) and 10 μL of 0.1 mg/mL quercitrin (quercetin 3-*O*-rhamnoside; flavonoid extraction standard) were added. Extractions were performed at 70 °C. Recovery of both extraction standards for all types of samples was consistently >90%. Sample extraction, processing, and LC and LC/MS analyses were performed using methods previously described with the following minor modifications (*37−39*). To each sample from *Eruca* and *Diplotaxis* species, 5 mg of tris(2-carboxyethyl) phosphine was added postfiltration into HPLC vials. It has previously been shown that tris-(2-carboxyethyl) phosphine reduces any oxidized glucosinolates formed as artifacts during extraction (*13*). To simultaneously measure a wide range of phytochemicals in a single run, the solvent system was modified from that previously reported. Solvent A = 0.1% v/v trifluoracetic acid in ultrapure water, and solvent B = 0.1% v/v trifluoroacetic acid in HPLC grade acetonitrile. Selected samples were also analyzed by electrospray mass spectrometry in positive ion (ES+) and in negative ion (ES−) modes using an Agilent HP1100 diode array/ binary pump HPLC system coupled to an Agilent benchtop SL mass spectrometer and also using the previously described conditions in combination with a Quattro LC/MS/MS system (*37−39*). Peak identifications were confirmed from retention time, UV spectral data, and MS data and where possible with direct comparison to pure standards. Abbreviations for the glucosinolate names have been used in the text and tables.

**Acid Hydrolysis of Flavonoids for Aglycone Identification.** Extracts of young leaves and flowers of each of the species were processed and hydrolyzed using previously described methods, and the aglycones released were compared with commercial standards of kaempferol, quercetin, isorhamnetin, and rhamnetin by LC/MS (*39*).

Data Treatment from Analyses. Tissues of each species over the full lifecycles were analyzed from triplicate, separate experiments. The glasshouse grown plants showed less than 1% difference in standard deviation (SD) for each compound measured (as μg or mg/g fresh weight) in the seedling studies, and less than 5% difference in SD for each compound measured (as μg or mg/g fresh weight) in adult plants (vegetative and reproductive tissues). The data for each compound in each tissue are presented as a letter code representing the mean concentration from the triplicate experiments.

RESULTS AND DISCUSSION

Various phytochemicals have been reported for rocket species, and examples of glucosinolates and phenolics previously identified are shown in **Figure 1**. The most commonly encountered commercial products from rocket species are the leaves, and photos of leaves of the two *Diplotaxis* and *Eruca* species used in this study are shown in **Figure 2**.
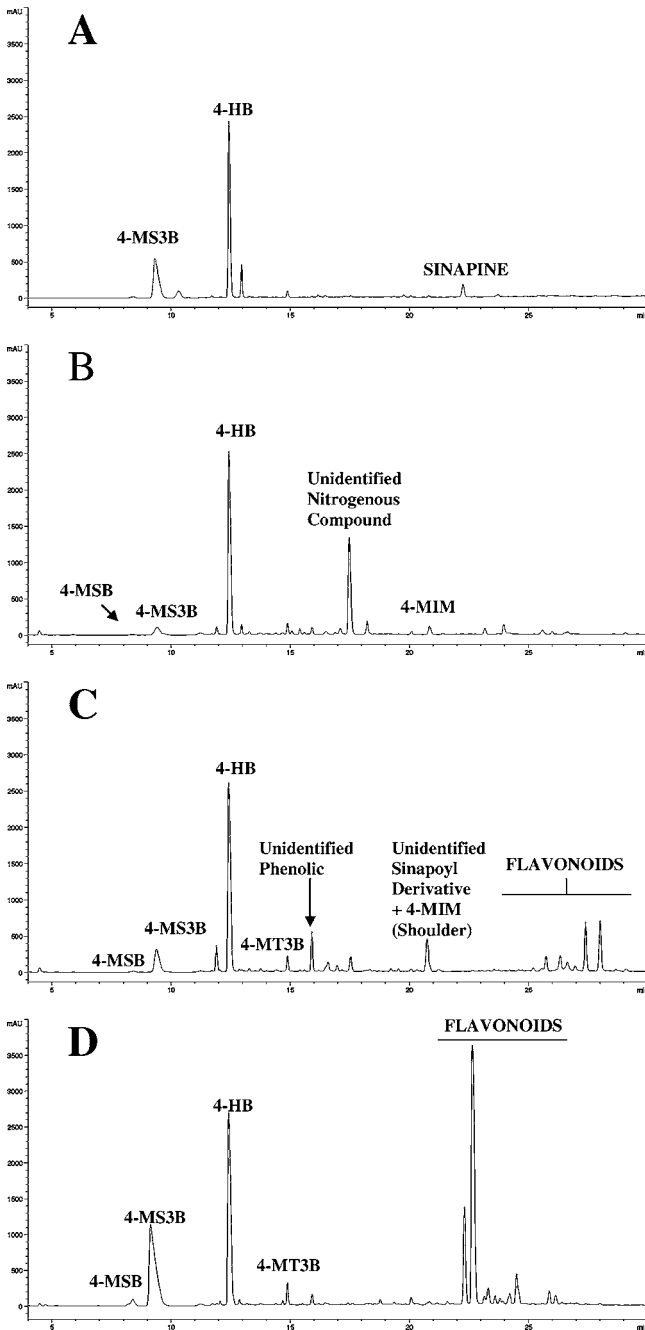
The phytochemical profiles and amounts of glucosinolates, flavonoids, and *N*-heterocycles in seeds of *E. sativa* L., from the different suppliers, varied very little suggesting a common original genetic origin for the majority of commercial seed (**Table 1** and **Figure 3A**). Fairway rocket had a phytochemical profile virtually identical to the *E. sativa* seeds. *B. orientalis* seed contained low levels of 4-MSB (<10 μg/g fresh weight) with predominant amounts of 4-methylsulfinyl-3-butenylglucosinolate (4-MS3B) (10−20 μg/g fresh weight) and 4-hydroxybenzylglucosinolate (4-HB) (30−40 μg/g fresh weight) (**Table 1** and **Figure 4A**). There were minimal differences in the phytochemical contents of seed of both *Diplotaxis* species with very little inter- or intraspecies differences in the phytochemicals measured (**Table 1** and **Figure 5A**). The major differences between the *Eruca* and the *Diplotaxis* species were the presence of the *N*-heterocycles in all *Eruca* (which are currently being purified for full identification by NMR), which were absent from both *Diplotaxis* species, and the presence of sinapine in both *Diplotaxis* species, which were absent from
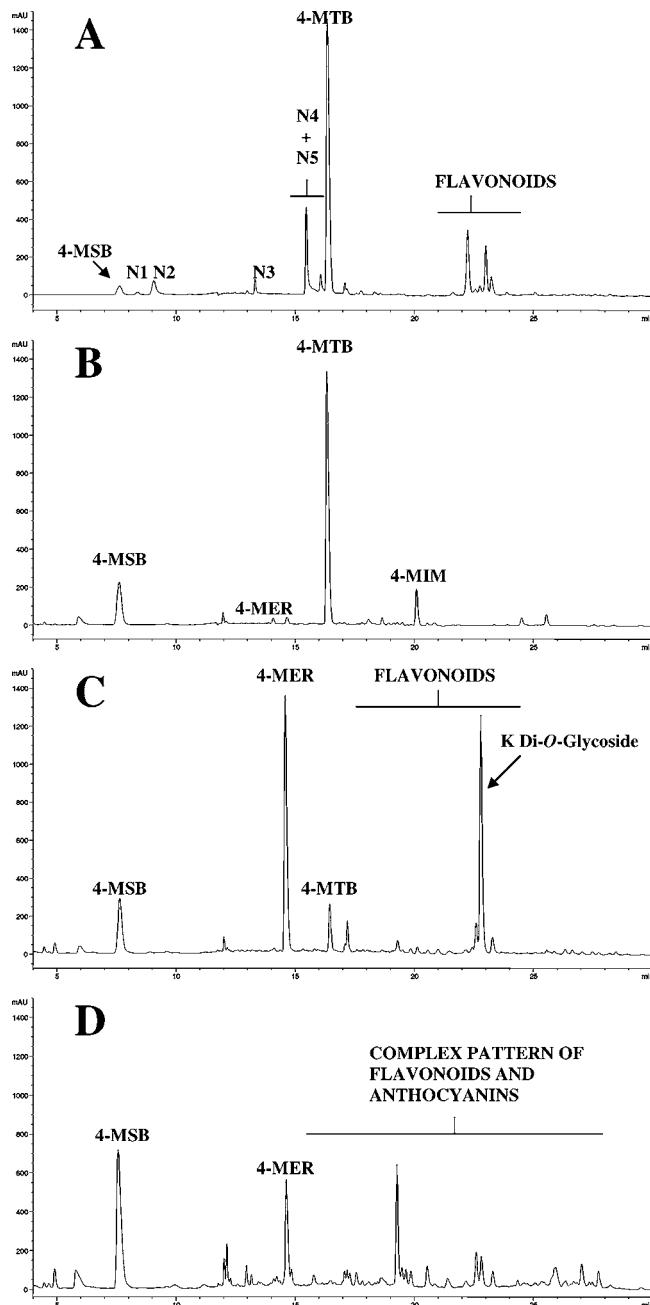
Figure 3. Example LC traces (227 nm) of seeds (**A**), roots (**B**), young leaves (**C**), and flowers (**D**) of *E. sativa* (grown from Nickersons seed). All chromatograms are in the same scale for comparison. Glucosinolate abbreviations are as used for the text and tables. N1−N5 = structurally related *N*-heterocycles. For the identitites of flavonoids, see the tables.
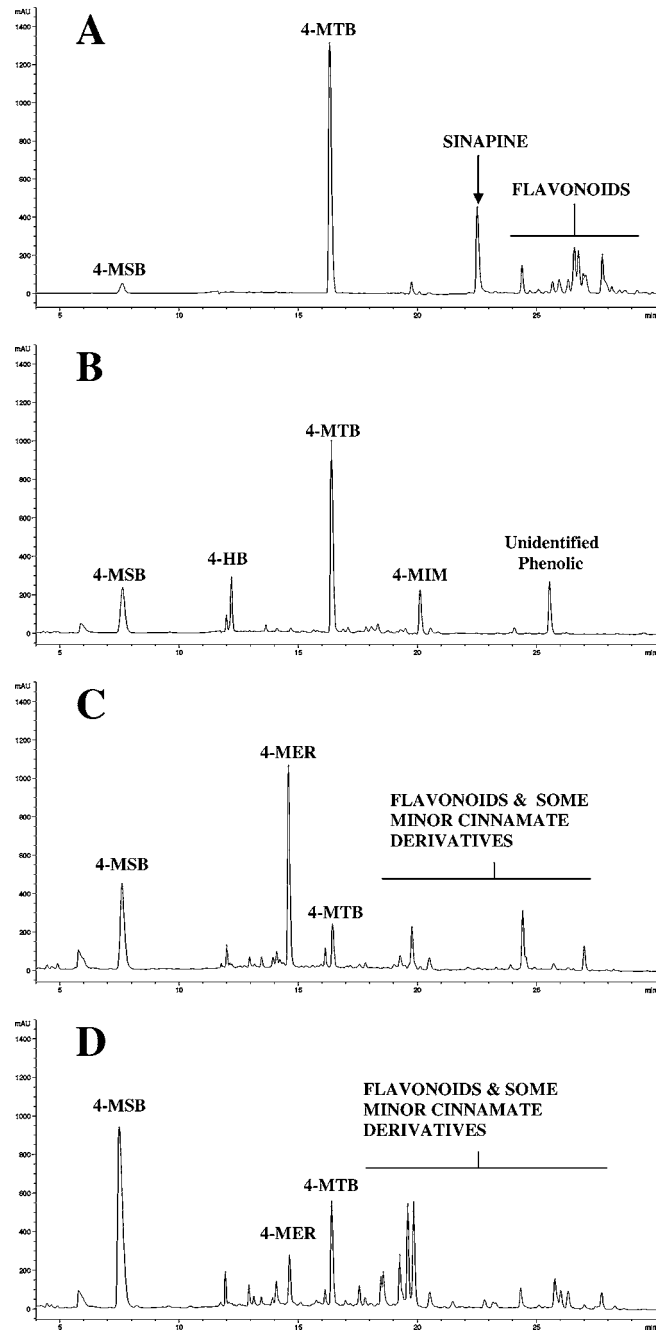
all *Eruca* seeds tested. Sinapine was also present in low levels in *B. orientalis* seed (**Table 2** and **Figure 4A**).

Sprouts (seedlings) of Capparales species, and specifically *Brassica* and closely related Brassicaceae, are commonly consumed throughout the world. On the basis of the minimal differences in seed profiles (**Table 2**), selected seed sources of the four species were used to produce seedlings and adult plants for further phytochemical analyses. Analyses of seedlings at DAG (days after germination) 3, 5, and 7 of *E. sativa* (from two sources), *Eruca* ssp. (one source), *D. erucoides* (two sources), and *D. tenuifolia* (one source) showed changes in profiles and content of glucosinolates (**Table 2**). Seeds of *B. orientalis* were not used in the seedling studies because of the

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Figure 4. Example LC traces (227 nm) of seeds (**A**), roots (**B**), young leaves (**C**), and flowers (**D**) of *B. orientalis* (grown from Future Foods seed). All chromatograms are in the same scale for comparison. Peak ID: Glucosinolate abbreviations are as used for the text and tables. For details of flavonoid identities, see the data tables.

Figure 5. Example LC traces (227 nm) of seeds (**A**), roots (**B**), young leaves (**C**), and flowers (**D**) of *D. erucoides* (grown from Seeds of Italy, Special Quality variety seed). The phytochemical profiles for the same tissues from *D. tenuifolia* were virtually identical to *D. erucoides* samples. All chromatograms are in the same scale for comparison. Peak ID: Glucosinolate abbreviations are as used for the text and tables. For details of flavonoid identities, see the data tables.

very long germination period to produce seedlings (>14 days) and also the hardness of the seed coat, making the sprouts less palatable as a potential human food. Unlike a recent paper (*40*), 4-MER was detected by LC/MS within 1 day from the onset of seed germination suggesting conversion of stored seed 4-MTB, although the levels remained low at DAGs 3, 5, and 7. It was not until the true leaves were formed that the levels increased. In seedlings, there was also a large reduction in 4-MTB and 4-MER that was predominantly due to dilution effects (75−85% of the reduction), but there was also some turnover of all glucosinolates (15−25%), possibly as a source of glucose and sulfate in the developing seedling. A similar pattern in the synthesis of 4-MER was observed in seedlings

of both *Diplotaxis* species with a lower dilution effect on 4-MTB (**Table 2**).

Analyses of glucosinolates in adult plant tissues and vegetative and floral tissues showed distinct differences in profiles (**Table 3**). In roots of all three adult *Eruca* species, the three aliphatic glucosinolates were present with 4-MTB being predominant. In leaves of the adult *Eruca* species, 4-MER was predominant, and in flowers, 4-MSB was predominant. In all adult *Eruca* tissues, no aromatic glucosinolates were detected and the levels of indole glucosinolates were very low (<100 μg/g fresh weight) (**Table 3**). In the roots of the two adult

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Table 2. Glucosinolates, Simple Phenolics, and *N*-Heterocycles in Seedlings of Rocket Species at 3, 5, and 7 DAG[a]

species (seed source)	seedling age (days after germination)	glucosinolates[b]					phenolics	*N*-heterocycles				
		4-MTB	4-MER	4-MSB	4-HB	4-MIM	sinapine	N1	N2	N3	N4	N5
E. sativa	DAG3	H	B	F		A		A	A	A	C	A
(E. W. Kings)	DAG5	G	B	E		A		A	A	A	B	A
	DAG7	E	A	B		A		A	A	A	B	A
E. sativa	DAG3	H	E	G		B		A	A	A	D	A
(Nickersons)	DAG5	D	C	C		A		A	A	A	B	A
	DAG7	B	B	B		A		A	A	A	A	A
Eruca ssp.	DAG3	H	E	G		B		A	A	A	D	A
(Unwins)	DAG5	D	C	C		A		A	A	A	B	A
	DAG7	B	B	B		A		A	A	A	A	A
D. erucoides	DAG3	I	A	G	A	A	F					
(Nickersons)	DAG5	G	A	D	A	A	C					
	DAG7	G	B	C	A	A	C					
D. erucoides	DAG3	H	A	F	A	A	F					
(Seeds of Italy)	DAG5	F	A	C	A	A	D					
	DAG7	E	B	B	A	A	C					
D. tenuifolia	DAG3	I	A	F	A	A	G					
(B&T World Seeds)	DAG5	H	A	E	A	A	F					
	DAG7	F	A	C	A	A	B					

[a] Concentrations were expressed as ranges of μg compound/g fresh weight. Fresh weight: blank cell, not detected by UV or MS; A, 1–100; B, 100–200; C, 200–300; D, 300–400; E, 400–500; F, 500–1000; G, 1000–2500; H, 2500–5000; and I, 5000–6000. Glucosinolates and sinapine were measured directly; *N*-heterocycles were expressed as indole-3-acetonitrile equivalents. Seedlings of *B. orientalis* were not evaluated due to their much longer germination time and the very hard seed coat (making them unsuitable as a human "sprout" food). [b] Glucosinolate abbreviations: 4-MIM, 4-methoxy-3-indolylmethyl.

Table 3. Glucosinolates in Roots, Young Leaves, and Flowers of Adult Rocket Species[a]

species (seed source for adult plant)	tissue[b]	glucosinolates[c]							
		4-MTB	4-MER	4-MSB	4-MT3B	4-MS3B	4-HB	4-HIM	4-MIM
E. sativa	roots	G	A	C				A	A
(E. W. Kings)	young leaves	B	E	C					A
	flowers		C	G					
E. sativa	roots	H	A	D				A	A
(Nickersons)	young leaves	C	F	D					A
	flowers		D	G					
Eruca ssp.	roots	C	B	B				A	A
(cv. Fairway)	young leaves	B	F	C					A
	flowers	A	D	H					
D. erucoides	roots	F	A	B			B		A
(Nickersons)	young leaves	B	G	D					A
	flowers	D	A	H					
D. erucoides	roots	F	A	C			B		A
(Seeds of Italy)	young leaves	C	F	E					A
	flowers	D	B	H					
D. tenuifolia	roots	E	A	D			B		A
(B&T)	young leaves	A	C	B					A
	flowers	D	B	H					
B. orientalis	roots			A		C	J		A
(Future Foods)	young leaves			A	B	D	J		A
	flowers			B	A	K	K		A

[a] No sinapine or *N*-heterocycles were detected in any of the tissues of adult plants; that is, these appear to be seed and seedling specific. Glucosinolate concentrations were expressed with a letter code representing the following ranges as μg potassium salt of intact glucosinolate/g fresh weight of tissue: blank cell, not detected by LC/UV or LC/MS; A, 1–100; B, 100–250; C, 250–500; D, 500–750; E, 750–1000; F, 1000–1500; G, 1500–2000; H, 2000–2500; J, 2500–3000; and K, 3000–3500. [b] Roots (total) and young leaves (counting from base of the plant; leaves 8–11 and leaf apices) were harvested from 36 day old *E. sativa*, 61 day old *Diplotaxis* (both species), and 78 day old *B. orientalis*. Whole flowers were collected from 76 day old *E. sativa*, 95 day old *Diplotaxis* (both species), and 105 day old *B. orientalis*. [c] Glucosinolate abbreviations: 4-MT3B, 4-methylthio-but-3-enyl; and 4-HIM, 4-hydroxy-3-indolylmethyl.

Diplotaxis species, there was a pattern similar to that found in roots of adult *Eruca* species but with the addition of 4-HB (**Table 3**).

In tissues of adult *B. orientalis*, the glucosinolates 4-MS3B and 4-HB were predominant; the latter was highest in all tissues (2500−3500 μg/g fresh weight) (**Table 3**). Leaves and flowers of *B. orientalis* also contained low levels of 4-methylthio-3-butenylglucosinolate, the probable precursor of 4-MS3B. 4-MS3B has previously been reported as a major glucosinolate in *Raphanus* ssp. (radish), and 4-HB is the major glucosinolate in *Sinapis alba* (white mustard) (*10*).

Flavonoids were present in all tissues of all of the species analyzed (**Tables 4** and **5**). Acid hydrolysis and LC/MS analyses showed that the core aglycones of the flavonoid glycosides in seeds, leaves, and flowers of *Eruca*, *Diplotaxis*, and *Bunias* were kaempferol, quercetin, and isorhamnetin (data not shown). Flavonoid concentrations were calculated using the calibrations curves for the commercial standards. The standard with the closest chemical structure to the previously unpurified flavonoid was used to express the new flavonoid as equivalents, e.g., quercetin monosinapoyl tri-*O*-glycoside was expressed as quercetin 3-*O*-sophoroside equivalents. Ideally, all of the new

Table 4. Flavonoids in Seeds and Seedlings and in Young Leaves and Flowers of Adult Plants of *E. sativa*, *Eruca* ssp. (Fairway), and *B. orientalis*[a]

species (seed source[b])	tissue	simple			acylated		
		mono-*O*-Gly	di-*O*-Gly	tri-*O*-Gly	mono-*O*-Gly	di-*O*-Gly	tri-*O*-Gly
E. sativa (1)	SEED	**C** (*Q*)	**G** (*Q*)	**F** (*Q*)		**C** (*Q-S*)	**J** (*Q-S*), **G** (*ISO-F*)
E. sativa (2)			**C** (*Q*)	**D** (*Q*)			**G** (*Q-S*), **E** (*ISO-F*)
Eruca ssp.			**D** (*Q*)	**D** (*Q*)			**H** (*Q-S*), **D** (*ISO-F*)
E. sativa (1)	DAG3	**A** (*Q*)	**B** (*Q*)	**B** (*Q*)		**A** (*Q-S*)	**C** (*Q-S*), **B** (*ISO-F*)
E. sativa (2)		**A** (*Q*)	**B** (*Q*)	**B** (*Q*)		**A** (*Q-S*)	**D** (*Q-S*), **C** (*ISO-F*)
Eruca ssp.		**A** (*Q*)	**B** (*Q*)	**B** (*Q*)		**B** (*Q-S*)	**C** (*Q-S*), **C** (*ISO-F*)
E. sativa (1)	DAG5	**A** (*Q*)	**B** (*Q*)	**A** (*Q*)		**A** (*Q-S*)	**C** (*Q-S*), **B** (*ISO-F*)
E. sativa (2)		**B** (*Q*)	**E** (*Q*)	**C** (*Q*)		**C** (*Q-S*)	**E** (*Q-S*), **D** (*ISO-F*)
Eruca ssp.		**A** (*Q*)	**B** (*Q*)	**B** (*Q*)		**A** (*Q-S*)	**D** (*Q-S*), **B** (*ISO-F*)
E. sativa (1)	DAG7	**A** (*Q*)	**B** (*Q*)	**B** (*Q*)		**A** (*Q-S*)	**C** (*Q-S*), **B** (*ISO-F*)
E. sativa (2)		**B** (*Q*)	**F** (*Q*)	**C** (*Q*)		**C** (*Q-S*)	**E** (*Q-S*), **D** (*ISO-F*)
Eruca ssp.		**A** (*Q*)	**B** (*Q*)	**C** (*Q*)		**A** (*Q-S*)	**C** (*Q-S*), **C** (*ISO-F*)
E. sativa (1)	YL		**D** (*K*), **A** (*Q*), **B** (*ISO*)	**A** (*K*), **A** (*Q*)			
E. sativa (2)			**D** (*K*), **A** (*Q*), **A** (*ISO*)	**A** (*K*), **A** (*Q*)			
Eruca ssp.			**D** (*K*), **A** (*Q*), **A** (*ISO*)	**A** (*K*), **A** (*Q*)			
B. orientalis			**A** (*K*)	**A** (*K*)		**A** (*K-AC*), **C** (*K-S*), **C** (*K-S/M*)	
E. sativa (1)	FLO	**B** (*K*), **B** (*ISO*)	**C** (*K*), **C** (*Q*), **C** (*ISO*)	**A** (*K*), **A** (*Q*), **B** (*ISO*)			
E. sativa (2)		**B** (*K*), **B** (*ISO*)	**B** (*K*), **B** (*Q*), **A** (*ISO*)	**A** (*K*), **A** (*Q*), **A** (*ISO*)			
Eruca ssp.		**A** (*K*), **A** (*ISO*)	**A** (*K*), **A** (*Q*), **A** (*ISO*)	**A** (*K*), **A** (*Q*), **A** (*ISO*)			
B. orientalis		**A** (*Q*)	**H** (*Q*), **C** (*ISO*)	**A** (*K*), **D** (*Q*)			

[a] Tissue abbreviations: YL, young leaves; and FLO, whole flowers. Flavonoid concentrations are expressed as ranges of μg flavonoid/g fresh weight of tissue where blank cell, not detected; A, 1–50; B, 50–100; C, 100–250; D, 250–500; E, 500–750; F, 750–1000; G, 1000–1500; H, 1500–2000; I, 2000–2500; and J, 2500–3000. The letters in italics in parentheses represent the core aglycones for the flavonoids and the acyl substitution: *K*, kaempferol; *Q*, quercetin; and *ISO*, isorhamnetin. Additional acylation code letters: *CO*, *p*-coumaroyl; *F*, feruloyl; *S*, sinapoyl; *M*, malonylated; and *AC*, unidentified acylation (possibly monodemethylated sinapic acid). Many of the polyglucosylated/acylated flavonoids existed as isomers; for details of retention times and complete MS data, see the Supporting Information. No significant amounts of flavonoids were detected in the roots of any of the species, and seeds of *B. orientalis* were essentially free of flavonoids. [b] Seed sources: *E. sativa* (1), E. W. Kings; *E. sativa* (2), Nickersons; and *Eruca* ssp., Unwins cv. Fairway.

flavonoids would have been purified to allow the production of specific calibration curves, but with the large number of new flavonoids, this was not possible. In seeds of *B. orientalis*, flavonoids were negligible (<1 μg/g fresh weight) and were not characterized further. In *Eruca* and *Diplotaxis* seeds, the flavonoids were present as complex polyglycosylated and/or polyglycosylated/acylated flavonols (**Tables 4** and **5**). In the two *Diplotaxis* species, the flavonoid profiles changed during seedling development; the major change was a dilution effect as the seedling grew (indicative of no de novo biosynthesis of flavonoids) (**Table 5**). In the two *Eruca* samples, two further flavonoids (quercetin 3-*O*-glucoside and quercetin monosinapoyl di-*O*-glycoside) were present 7 days postgermination; either precursors or degradation products of some of the more complex flavonoids that were already present (**Table 4**). By DAG 7, the first pair of true leaves (5−7 mm long) was forming in the *Eruca* seedlings whereas in *Diplotaxis* the true leaf primordia were only just visible (cotyledons fully expanded).

In the roots of adult plants of *B. orientalis*, *E. sativa* and *Eruca* ssp. Fairway, *D. erucoides*, and *D. tenuifolia*, the levels of flavonoids were very low (<1 μg/g fresh weight) and were not further characterized or quantified. In adult plant tissues of the *Eruca* species, unlike a previous report on leaf flavonoids

(*19*), no acylated flavonoids were detected (**Table 4**). In *Eruca* leaves, a single kaempferol di-*O*-glycoside was predominant with a kaempferol di-*O*-glycoside isomer, a quercetin di-*O*-glycoside and a quercetin tri-*O*-glycoside, and a isorhamnetin di-*O*-glycoside. In flowers, the profile changed and was essentially an equal mix of mono-*O*-, di-*O*-, and tri-*O*-glycosides of kaempferol, quercetin, and isorhamnetin (**Table 4**). It is likely that the quercetin tri-*O*-glycoside and quercetin monosinapoyl tri-*O*-glycoside identified in this study are those quercetin glucosides previously identified (*19*). The previously reported leaf quercetin disinapoyl tri-*O*-glycoside was not found in any *Eruca* tissues in the current study (*19*). In addition, the identification of other flavonoid glycosides in *Eruca* leaves in the current study, and specifically the predominant kaempferol di-*O*-glycoside, suggests that the leaves analyzed in the earlier study were not *E. sativa* or were an Italian ecotype with a very different leaf flavonoid profile to common commercially available *E. sativa*. Two other studies have reported the kaempferol content of leaves of *E. vesicaria* ssp. *sativa* from aglycone analysis (40 μg/g fresh weight) and also individual partially identified glycosides (one predominant and two major kaempferol glycosides and minor amounts of quercetin and isorhamnetin

Table 5. Flavonoids in Seeds and Seedlings and in Young Leaves and Flowers of Adult Plants of *D. erucoides* and *D. tenuifolia*[a]

species (seed source[a])	tissue	simple				acylated	
		mono-*O*-Gly	di-*O*-Gly	tri-*O*-Gly	tetra-*O*-Gly	di-*O*-Glc	tri-*O*-Gly
D. erucoides (1)	SEED	**G** (*ISO*)	**C** (*Q*)	**G** (*Q*)		**L** (*Q-F*), **G** (*Q-S*) **C** (*ISO-C*), **H** (*ISO-F*), **E** (*ISO-S*)	
D. erucoides (2)		**G** (*ISO*)	**C** (*Q*)	**G** (*Q*)		**L** (*Q-F*), **F** (*Q-S*) **B** (*ISO-C*), **H** (*ISO-F*), **E** (*ISO-S*)	
D. tenuifolia		**G** (*ISO*)	**C** (*Q*)	**G** (*Q*)		**J** (*Q-F*), **G** (*Q-S*) **C** (*ISO-C*), **H** (*ISO-F*), **E** (*ISO-S*)	
D. erucoides (1)	DAG3	**D** (*ISO*)	**A** (*Q*)	**D** (*Q*)		**D** (*Q-F*), **D** (*Q-S*) **A** (*ISO-C*), **D** (*ISO-F*), **C** (*ISO-S*)	
D. erucoides (2)		**D** (*ISO*)	**A** (*Q*)	**D** (*Q*)		**E** (*Q-F*), **E** (*Q-S*) **A** (*ISO-C*), **D** (*ISO-F*), **C** (*ISO-S*)	
D. tenuifolia		**D** (*ISO*)	**A** (*Q*)	**D** (*Q*)		**E** (*Q-F*), **D** (*Q-S*) **A** (*ISO-C*), **D** (*ISO-F*), **C** (*ISO-S*)	
D. erucoides (1)	DAG5	**C** (*ISO*)	**A** (*Q*)	**C** (*Q*)		**D** (*Q-F*), **C** (*Q-S*) **A** (*ISO-C*), **C** (*ISO-F*), **C** (*ISO-S*)	
D. erucoides (2)		**C** (*ISO*)	**A** (*Q*)	**D** (*Q*)		**C** (*Q-F*), **C** (*Q-S*) **A** (*ISO-C*), **C** (*ISO-F*), **B** (*ISO-S*)	
D. tenuifolia		**C** (*ISO*)	**A** (*Q*)	**D** (*Q*)		**D** (*Q-F*), **C** (*Q-S*) **A** (*ISO-C*), **C** (*ISO-F*), **B** (*ISO-S*)	
D. erucoides (1)	DAG7	**B** (*ISO*)	**A** (*Q*)	**B** (*Q*)		**C** (*Q-F*), **B** (*Q-S*) **A** (*ISO-C*), **B** (*ISO-F*), **A** (*ISO-S*)	
D. erucoides (2)		**C** (*ISO*)	**A** (*Q*)	**C** (*Q*)		**C** (*Q-F*), **C** (*Q-S*) **A** (*ISO-C*), **C** (*ISO-F*), **A** (*ISO-S*)	
D. tenuifolia		**C** (*ISO*)	**A** (*Q*)	**B** (*Q*)		**C** (*Q-F*), **C** (*Q-S*) **A** (*ISO-C*), **B** (*ISO-F*), **A** (*ISO-S*)	
D. erucoides (1)	YL			**B** (*Q*)	**A** (*Q*)	**A** (*Q-S*)	**C** (*Q-S*)
D. erucoides (2)				**B** (*Q*)	**A** (*Q*)	**A** (*Q-S*)	**C** (*Q-S*)
D. tenuifolia				**A** (*Q*)	**A** (*Q*)	**A** (*Q-S*)	**B** (*Q-S*)
D. erucoides (1)	FLO	**A** (*ISO*)	**B** (*K*) **A** (*Q*) **B** (*ISO*)	**A** (*Q*) **C** (*ISO*)	**A** (*Q*)		
D. erucoides (2)		**A** (*ISO*)	**B** (*K*) **A** (*Q*) **C** (*ISO*)	**A** (*Q*) **D** (*ISO*)	**A** (*Q*)		
D. tenuifolia		**A** (*ISO*)	**A** (*K*) **B** (*Q*) **C** (*ISO*)	**B** (*Q*) **C** (*ISO*)	**A** (*Q*)		

[a] Tissue abbreviations: YL, young leaves; and FLO, whole flowers. Flavonoid concentrations were expressed as ranges of μg flavonoid/g fresh weight of tissue where: blank cell, not detected; A, 1–50; B, 50–100; C, 100–250; D, 250–500; E, 500–750; F, 750–1000; G, 1000–1500; H, 1500–2000; I, 2000–2500; J, 2500–3000; and L, 3000–3500. Letters in italics in parentheses represent the core aglycones for the flavonoids and the acyl substitutions: *K*, kaempferol; *Q*, quercetin; and *ISO*, isorhamnetin. Additional acylation code letters: *C*, *p*-coumaroyl; *CF*, caffeoyl; *F*, feruloyl; and *S*, sinapoyl. [b] Seed sources: *D. erucoides* (1), Nickersons; *D. erucoides* (2), Seeds of Italy (special quality); and *D. tenuifolia*, B&T World Seeds.

glycosides) (*21, 22*). Both of these previous results are comparable to those of the current study.

In leaves of *B. orientalis*, a kaempferol monosinapoyl di-*O*-glycoside and a kaempferol monomalonyl/monosinapoyl di-*O*-glycoside were predominant; a kaempferol di-*O*-glucoside and tri-*O*-glucoside were also present (**Table 4**). In addition, in leaves, there was another acylated kaempferol di-*O*-glucoside for which the acyl group was tentatively identified as a demethylated sinapic acid.

In leaves of both *D. erucoides* and *D.tenuifolia*, a quercetin monosinapoyl tri-*O*-glycoside was predominant with a quercetin tri-*O*-glycoside, a quercetin tetra-*O*-glycoside, and a quercetin monosinapoyl di-*O*-glycoside (**Table 5**). Flowers of the *Diplotaxis* species did not contain any acylated flavonoids but consisted of a mix of kaempferol di-*O*-glycoside, quercetin di-*O*-, tri-*O*-, and tetra-*O*-glycosides, and isorhamnetin mono-*O*-, di-*O*-, and tri-*O*-glycosides (**Table 5**). A previous qualitative study of flavonoids (as acid-released aglycones) in *D. tenuifolia* reported kaempferol, quercetin, and isorhamnetin in leaves and quercetin and isorhamnetin in petals (*42*). In this study, no isorhamnetin glycosides were found in leaves, and additionally, flowers had a kaempferol di-*O*-glycoside (**Table 5**).

Concentrations and structural classes of flavonoids are affected by various stresses such as light, nutrient supply,

growing conditions, and especially UV light (*43, 44*). Therefore, some of the differences between data presented in this paper and previous reports could be due to differences in light quality and intensity and growing conditions.

Anthocyanins were only detected in the flowers of *E. sativa* (standard and Fairway varieties) and were not detected in any other tissues of unstressed *E. sativa* nor were they present in any of the tissues of the *Diplotaxis* species or *B. orientalis*. However, at the base of stems and in old leaves of very old, postpod formation, in *E. sativa*, a distinct purple coloration was observed indicative of stress-induced anthocyanin synthesis; these tissues were not analyzed. The flowers of *E. sativa* had a surprisingly complex anthocyanin profile, and they were not fully characterized by LC/MS. Total amounts, expressed as μg cyanidin 3-*O*-glucoside equivalents/g fresh weight, were 5254 ± 29 (for *E. sativa* produced from E. W. Kings seed), 4602 ± 34 (for *E. sativa* produced from Nickersons seed), and 460 ± 32 (for *Eruca* ssp. produced from Unwins cv. Fairway seed). The anthocyanin HPLC profile and the UV−visible spectra for each anthocyanin can be found in the Supporting Information.

Polyglycosylated and polyglycosylated/acylated flavonoids are common in the Brassicaceae, and there is considerable data on *Brassica* flavonoids, especially for broccoli (*Brassica oleracea* var. *italica*) (*45−54*). However, for many other food

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and herb Brassicaceae and related Capparales, there are often very little or no data, thus making comparisons on dietary flavonoid intake for these different species very difficult; often, the data are qualitative and rarely quantitative. It is known that the increase in glycosylation and acylation makes the flavonoids much less bioavailable as compared with the simple glucosylated flavonoids found in other foods, e.g., onions. Some of the limiting factors are the substrate specificity of lactase phlorizonhydrolase, the specificity of the uptake transporter sodium-dependent glucose transporter 1, and finally the specificity of the enterocyte cytosolic β-glucosidase (*16*). Therefore, the complex flavonoids found in the rocket species are likely to have a lower bioavailability due to the release of the core aglycone being dependent on the colonic microflora as is the case for the complex polymeric flavan-3-ols (procyanidins) (*14*, *15*).

Hydroxycinnamate derivatives of either the disaccharide gentiobiose or quinic acid (chlorogenic acids) were only detected, at very low levels, by LC/MS in leaf and floral tissues of *Bunias*, *Diplotaxis*, and *Eruca* species. Chlorogenic acids are found in many plant families with widely ranging concentrations. Common dietary rich sources of chlorogenic acids include coffee, artichokes (*Cynara scolymus* L.), plums (*Prunus domestica* L.), and pears (*55*−*57*). Within the Brassicaceae, and specifically *Brassica* species, significant levels of chlorogenic acids have previously been reported for broccoli (*55*, *58*). Chlorogenic acids have also been found in leafy *Brassica* species, e.g., kale (6−120 μg/g), cabbage (up to 104 μg/g), and Brussels sprouts (up to 37 μg/g) (*55*). In broccoli florets at harvest, total chlorogenics were 86.8 μg/g fresh weight and total feruloyl/sinapoyl-gentiobiose esters were 151.5 μg/g fresh weight (*58*). Chlorogenic acids have also been found in other Capparales species, e.g., *Moringa* (Moringaceae) (*37*). In addition, the previously reported high levels of free hydroxycinnamates and sinapoyl-glucose in *B. orientalis* leaf tissues were not found in this study (*23*).

In the most commonly consumed parts of both *Eruca* and *Diplotaxis* species, the leaves, the predominant glucosinolate was commonly 4-MER. Upon hydrolysis, this glucosinolate produces a very volatile and pungent isothiocyanate with a very characteristic flavor (*13*). In neither the *Eruca* nor *Diplotaxis* samples were significant levels of the recently reported disulfide glucosinolates detected (*59*) and derived from 4-MER by either reaction with a second molecule of this glucosinolate (to produce a dimer) or with other endogenous thiols such as thioglucose and glutathione to produce mixed disulfides. On the basis of data presented in this paper and previous findings, this suggests that the formation of these disulfide glucosinolates is an artifact of the extraction processes used (*13*). Thus, the supposedly new *Eruca* glucosinolate recently reported (*59*) is most likely an artifact and is unlikely to exist in planta unless there is extensive oxidative stress. In initial studies, without the addition of tris-(2-carboxyethyl) phosphine as a reducing agent, it was found that in addition to the 4-MER dimer, there were some minor intact disulfide glucosinolates (Bennett, R. N. Unpublished data). One had an $[M - H]^- = 600$ for the intact glucosinolate corresponding to the analogous $[M + H]^+ = 522$ desulfoglucosinolate reported (*59*). In addition, a smaller peak corresponding to a mixed disulfide with endogenous glutathione was also identified (Bennett, R. N. Unpublished data). Thus, without postextraction reducing conditions, the 4-MER will readily oxidize, reacting with itself and any other in planta thiols nonenzymatically forming these various disulfide artifacts.

Raw tissues of rocket species, and particularly *Eruca* and *Diplotaxis* species, have considerable potential as functional foods at both the seedling and the adult plant stages (leaves and flowers) for delivering significant levels of bioactive compounds to humans and especially glucosinolate-derived isothiocyanates and various vitamins. However, the very distinctive flavors and odors of these species, especially the leaves, means they will probably not be as readily consumed as the analogous tissues of broccoli and radish. Leaves of *B. orientalis*, reported as an alternative leafy vegetable to spinach, have a bitter flavor, even when parboiled; thus, it would be of limited appeal.

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