Betacyanins and Phenolic Compounds from *Amaranthus spinosus* L. and *Boerhavia erecta* L.

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Stem bark extracts of *Boerhavia erecta* L. (erect spiderling) and *Amaranthus spinosus* L. (spiny amaranth), two wild growing weed plants used in traditional African medicine, were characterized with respect to their phenolic profile including the betalains. While the main betalains in *A. spinosus* were identified as amaranthine and isoamaranthine, the major betacyanins in *B. erecta* were betanin, isobetanin together with neobetanin. The latter showed higher betalain concentrations amounting to 186 mg/100 g, while the former contained 24 mg betacyanins in 100 g of the ground plant material. Extracts of *A. spinosus* were found to contain hydroxycinnamates, quercetin and kaempferol glycosides, whereas catechins, procyanidins and quercetin, kaempferol and isorhamnetin glycosides were detected in *B. erecta*. The amounts of these compounds ranged from 305 mg/100 g for *A. spinosus* to 329 mg/100 g for *B. erecta*.

Key words: Amaranthus spinosus, *Boerhavia erecta*, Phenolics

Introduction

Amaranthus sp. (Amaranthaceae) and *Boerhavia* sp. (Nyctaginaceae) are used in tropical and subtropical countries for human nutrition both as vegetables (*Amaranthus*, *Boerhavia*) and grains (*Amaranthus*) but also as animal feed (Berghofer and Schoenlechner, 2002; Miralles *et al.*, 1988). Furthermore, members of both genera are popular medicinal plants to treat several ailments such as malaria, hepatic disorders, jaundice, scanty urine or to cure wounds (Berghofer and Schoenlechner, 2002; Samy *et al.*, 1999; Srivastava *et al.*, 1998). Various substance classes have been reported in root, leaf and stem tissues in members of the genera *Boerhavia* and *Amaranthus*, *i.e.* alkaloids, amino acids, minerals, carbohydrates such as sugars and starch, lipids, saponins, carotenoids, tannins, and other phenolic substances (Braun-Sprakties, 1992; Edeoga and Ikem, 2002; Srivastava *et al.*, 1998; Teutonico and Knorr, 1985). However, studies on the compound profile of the wild growing weeds *B. erecta* L., and *A. spinosus* L. are still lacking. While previous reports suggested amaranthine and its epimer as major betalains in *A. spinosus* seeds (Cai *et al.*, 2001), the betalains in *B. erecta* have so far not been investigated. Furthermore, to the best of our knowledge, information on the phenolic constituents of stem bark extracts of the two species is still lacking. Therefore, the present investigation aimed at quantitative and qualitative determination of polyphenolics and betalains from stem bark extracts that are used traditionally in West Africa for medicinal and food colouring purposes.

Material and Methods

Plant material

The stems of *Boerhavia erecta* L. (Nyctaginaceae) and *Amaranthus spinosus* L. (Amaranthaceae) were collected in January 2002 in the former experimental garden of the Institute for Rural Development (IDR), University of Ouagadougou, Ouagadougou, Province of Kadiogo, Burkina Faso (West Africa). Voucher specimens (Hilou.A.01 for *Amaranthus spinosus* L. and Hilou.A.02 for *Boerhavia erecta* L.) were deposited at the Herbarium of the Laboratory of Ecology and Plant Biology, UFR/SVT, University of Ouagadougou. The plants were identified by the botanist Prof. Millogo/

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Rasolodimby Jeanne. The barks were removed from the stems with a knife, dried in the laboratory at 30 °C for 36 h, and pulverized in a laboratory mortar. The so obtained ground materials were stored out of light and dampness.

Solvents and reagents

All reagents and solvents used were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Deionized water was used throughout. Catechin, chlorogenic acid, isorhamnetin 3-*O*-glucoside, isorhamnetin 3-*O*-rutinoside, procyanidin B1, quercetin 3-*O*-glucoside and quercetin 3-*O*-rutinoside were from Extrasynthese (Lyon, France); *p*-coumaric acid, ferulic acid and kaempferol were purchased from Roth (Karlsruhe, Germany).

Extraction and purification of extracts

To exactly 5.00 g of ground plant material seasand was added before homogenization in a mortar to ease extraction with 25 ml purified water. The betalains and phenolics obtained were separated from the solid material by passing the slurry through a Büchner funnel with a filter paper (Schleicher & Schuell, Dassel, Germany). The pH of the resulting solutions was 5.2 for *Boerhavia erecta* and 5.4 for *Amaranthus spinosus*, respectively. From an aliquote of these crude extracts, slightly concentrated *in vacuo* at room temperature, betalain quantifications and colour determinations were carried out. In addition HPLC runs to monitor the betalain pattern of tenfold concentrated crude extracts were performed. The remaining filtrates were taken for further workup. The betalains were separated from the other phenolic compounds after fractionating three times against ethyl acetate, previously adjusted to pH 1.5 with TFA. While the aqueous phase contained the betalains, the phenolic compounds were in the lipophilic phase. Subsequently, both fractions were treated separately. The coloured fraction was adjusted to pH 6 with aqueous ammonia (1.5 M NH_4OH) to ensure optimum betalain stability and then concentrated *in vacuo* at room temperature to reach concentrations sufficient for HPLC-MS analyses. Additionally, through removal of the colourless phenolics, improved mass spectra for betalains were obtained because potential co-elution was thus prevented. The phenolics were concentrated by evaporating ethyl acetate *in vacuo* until dryness and then dissolved in 5 ml methanol for further analyses. All experiments were performed in duplicate.

LC analyses of phenolic substances

The separation of phenolic compounds was performed on an Agilent HPLC series 1100 (Agilent, Waldbronn, Germany) equipped with ChemStation software, a degasser model G1322A, a binary gradient pump model G1312A, a thermoautosampler model G1329/1330A, a column oven model G1316A, and a diode array detector model G1315A. The column used was an Aqua 5 μm C_{18} (250 × 4.6 mm I.D.) from Phenomenex (Torrance, CA, USA) and a security guard C_{18} ODS (4 × 3.0 mm I.D.), operated at a temperature of 25 °C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and of 0.5% acetic acid in water and acetonitrile (50:50, v/v; eluent B). The gradient program was as follows: 10% B to 30% B (10 min), 30% B isocratic (5 min), 30% B to 46.5% B (30 min), 46.5% B to 100% B (5 min), 100% B isocratic (5 min), 100% B to 10% B (2 min) for *A. spinosus* and 10% B to 30% B (15 min), 30% B isocratic (5 min), 30% B to 55% B (40 min), 55% B to 100% B (15 min), 100% B isocratic (8 min), 100% B to 10% B (2 min) for *B. erecta*. The injection volume for all samples ranged from 2 to 10 μl. Simultaneous monitoring was performed at 280 nm (flavanols), 320 nm (hydroxycinnamic acids) and 370 nm (flavonols) at a flow rate of 1 ml/min. Spectra were recorded from 200 to 600 nm.

Quantification of phenolic substances

Individual phenolic compounds were quantified using calibration curves of the respective standards. Quantification of all other substances was carried out using calibration curves of related reference compounds and a molecular weight correction factor according to Chandra *et al.* (2001).

LC analyses of betalains

HPLC analyses were carried out with an HPLC system (Merck, Darmstadt, Germany) equipped with an auto sampler L-7200, an interface module D-7000, a pump L-7100, a column-oven L-7350 with Peltier cooling module, and a diode array detector L-7450A. Separation of all betalains was achieved at 25 °C and a flow rate of 1 ml/min using an analytical scale (250 x 3 mm I.D.) Luna $C_{18(2)}$-

reversed phase column with a particle size of 5 μm (Phenomenex, Torrance, CA, USA), fitted with a security guard C_{18} ODS (4 × 3.0 mm I.D.). Eluent A consisted of 1% (v/v) formic acid in water and a mixture of acetonitrile/water (80:20, v/v) was used as eluent B. Starting with 5% B in A at 0 min, a linear gradient was followed to 33% B in A at 35 min. Monitoring of betalains was performed at 476 nm for betaxanthins and at 538 nm for betacyanins.

Quantification of betalains

Quantification of betacyanins without prior removal of phenolic compounds was carried out using a UV-Vis spectrometer (Perkin-Elmer, Überlingen, Germany) equipped with a UV-Vis (UVWinLab V 2.85.04) software. Samples were diluted in a 0.05 M phosphate buffer (pH 6.5) as previously described (Stintzing *et al.*, 2003) using the extinction coefficients of betanin (ε = 60000 l/mol·cm; λ = 538 nm; molecular weight = 550; Wyler and Meuer, 1979) and neobetanin (ε = 18200 l/mol·cm; λ = 476 nm; molecular weight = 548; Wyler and Meuer, 1979) for *Boerhavia erecta* and of amaranthine (ε = 56600 l/mol·cm; λ = 538 nm; molecular weight = 726; Piattelli *et al.*, 1969) for *Amaranthus spinosus*, respectively. The obtained values were corrected by the respective chromatogram areas at 538 nm and 476 nm, respectively (Stintzing *et al.*, 2003).

Colour analyses of plant extracts

The betalain solutions directly obtained after extraction of stem bark material were diluted in the same buffer as used for the quantification experiments to reach an absorption value of $0.85 \leq A \leq 0.95$ at λ_{max}. L*a*b* colour parameters were assessed with the same UV-Vis spectrometer as described above equipped with a colour (Wincol V 2.05) software (Perkin-Elmer, Norwalk, CT, USA). Using illuminant D_{65} and 10° observer angle, metric chroma (C*) and hue angle (h°) were obtained by the transformation of a* and b* cartesian coordinates into polar ones according to $C^* = (a^{*2} + b^{*2})^{1/2}$ and h° = arctan (b*/a*).

LC-MS analyses

LC-MS analyses were performed with the HPLC system described for the LC analyses of phenolic substances. This HPLC system was connected in series with a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an ESI source. Negative ion MS was performed for phenolic compounds (range: *m/z* 50–1000) except the betalains (range: *m/z* 50–1000) which were characterized in the positive ionization mode. Nitrogen was used as the dry gas at a flow rate of 12.0 l/min and a pressure of 70.0 psi. The nebulizer temperature was set at 365 °C. Using helium as the collision gas (4.1×10^{-6} mbar), collision-induced dissociation spectra were obtained with a fragmentation amplitude of 1.2 V (MS/MS) for both phenolics and betalains and of 1.6 V (MS^3) for phenolics. Chlorogenic acid, quercetin 3-*O*-glucoside and procyanidin B1 were used for the optimization of the ionization parameters for the LC-MS analyses.

Results and Discussion

Phenolics

The characteristic data and contents of phenolic acids and flavonoids detected in extracts of *Amaranthus spinosus* and *Boerhavia erecta* are shown in Table I. As can be seen, six hydroxycinnamate derivatives were detected in *A. spinosus*, with a caffeoylquinic acid being the predominant compound. The characterization of the hydroxycinnamates was based on UV and mass spectral data, and by comparison of these data with those reported by Clifford *et al.* (2003) who established a hierarchical scheme for the identification of hydroxycinnamic acid derivatives. Since the fragmentation patterns in the MS^2 and MS^3 events did not match those given by Clifford *et al.* (2003), the presence of 3-, 4-, and 5-monoacylchlorogenic acids could be excluded. Furthermore, the retention times of a mixture of 3-, 4-, and 5-chlorogenic acids prepared by isomerization of 5-caffeoylquinic acid in a phosphate buffer according to Brandl and Herrmann (1983) were different from those of the caffeoylquinic acids detected in *A. spinosus* (data not shown). Therefore, it may be assumed that these compounds are either 1-monoacylchlorogenic acids or contain less common quinic acid moieties such as *muco*-quinic or *iso*-quinic acids (Clifford, 2003). Another possibility would be the presence of *cis*-cinnamates which occur as artefacts from their respective *trans*-isomers (Clifford, 2003).

Furthermore, four flavonol glycosides were detected, two of which were readily identified as quercetin (Q)-3-*O*-rutinoside and Q-3-*O*-gluco-

Table I. Characteristic data and contents of phenolic acids and flavonoids from *Amaranthus spinosus* and *Boerhavia erecta*.

	Retention time [min]	HPLC-DAD λ_{max} [nm]	$[M-H]^-$ *m/z*	HPLC-ESI(-)-MS^n experiment *m/z* (% base peak)	Content [mg/100 g]
Amaranthus spinosus					
Caffeoylquinic acid	15.6	243, 302sh, 327	353	– MS^2 [353]: 191 (62), 173 (100), 155 (4), 111 (15) – MS^3 [353 → 173]: 155 (23), 129 (6), 111 (100)	109.2 ± 15.6
Caffeoylquinic acid	16.1	234, 314	353	– MS^2 [353]: 191 (60), 173 (100), 155 (7), 111 (18) MS^3 [353 → 173]: 155 (24), 129 (10), 111 (100)	5.5 ± 0.5
Coumaroylquinic acid	21.3	233, 301sh, 314	337	– MS^2 [337]: 173 (100), 155 (4), 111 (18) – MS^3 [337 → 173]: 155 (25), 111 (100)	54.6 ± 6.0
Coumaroylquinic acid	22.6	232, 310	337	– MS^2 [337]: 191 (7), 173 (100), 155 (5), 111 (19) – MS^3 [337 → 173]: 155 (23), 111 (100)	17.5 ± 2.0
Feruloylquinic acid	24.2	239, 302sh, 328	367	– MS^2 [367]: 173 (100), 155 (10), 111 (31) – MS^3 [367 → 173]: 155 (25), 111 (100)	57.4 ± 5.5
Feruloylquinic acid	25.0	234, 322	367	– MS^2 [367]: 173 (100), 155 (8), 111 (25) – MS^3 [367 → 173]: 155 (27), 111 (100)	6.5 ± 0.2
Quercetin diglycoside	25.9	231, 257, 264sh, 300sh, 357	609	– MS^2 [609]: 301 (100), 300 (37) – MS^3 [609 → 301]: 179 (96), 151 (100) – MS^3 [609 → 300]: 271 (100), 255 (87)	1.9 ± 0.3
Quercetin 3-*O*-rutinoside	26.6	231, 256, 264sh, 302sh, 354	609	– MS^2 [609]: 301 (100), 300 (21) – MS^3 [609 → 301]: 179 (92), 151 (100) – MS^3 [609 → 300]: 271 (100), 255 (66)	36.4 ± 9.8
Quercetin 3-*O*-glucoside	28.0	231, 256, 263sh, 302sh, 354	463	– MS^2 [463]: 301 (100), 300 (15) – MS^3 [463 → 301]: 179 (86), 151 (100) – MS^3 [463 → 300]: 271 (100), 255 (70)	9.0 ± 1.9
Kaempferol diglycoside	33.5	231, 265, 300sh, 348	593	– MS^2 [593]: 285 (100), 284 (6) – MS^3 [593 → 285]: 267 (25), 257 (100), 241 (39), 229 (47), 213 (37), 197 (24), 163 (24) – MS^3 [593 → 284]: 255 (100)	7.0 ± 1.8
Boerhavia erecta					
Procyanidin B1	10.4	233, 279	577	– MS^2 [577]: 559 (31), 451 (30), 425 (100), 407 (71), 289 (21)	7.8 ± 0.5
Catechin	12.9	233, 279	289	– MS^2 [289]: 245 (100), 205 (33), 203 (14), 179 (14) – MS^3 [289 → 245]: 227 (17), 203 (100), 188 (30), 187 (14), 175 (14), 161 (25)	19.5 ± 0.2
Procyanidin B2	14.6	232, 280	577	– MS^2 [577]: 559 (28), 451 (40), 425 (100), 407 (58), 289 (17)	9.3 ± 0.6
Epicatechin	17.3	232, 280	289	– MS^2 [289]: 245 (100), 205 (30), 203 (14), 179 (17) – MS^3 [289 → 245]: 227 (28), 203 (100), 188 (15), 187 (14), 175 (20), 161 (26)	7.0 ± 0.5
Dimeric procyanidin	19.7	232, 279	577	– MS^2 [577]: 559 (19), 451 (14), 425 (100), 407 (75), 289 (10)	19.2 ± 0.7
Quercetin diglycoside	29.6	230, 255, 264sh, 298sh, 354	609	– MS^2 [609]: 301 (100), 300 (54) – MS^3 [609 → 301]: 179 (100), 151 (93) – MS^3 [609 → 300]: 271 (100), 255 (57)	0.6 ± 0.2
Quercetin 3-*O*-rutinoside	30.3	234, 256, 263sh, 301sh, 353	609	– MS^2 [609]: 301 (100), 300 (27) – MS^3 [609 → 301]: 179 (100), 151 (83) – MS^3 [609 → 300]: 271 (100), 255 (46)	133.4 ± 8.1
Quercetin 3-*O*-glucoside	31.8	231, 256, 263sh, 303sh, 354	463	– MS^2 [463]: 301 (100), 300 (16) – MS^3 [463 → 301]: 179 (100), 151 (88) – MS^3 [463 → 300]: 271 (100), 255 (57)	10.8 ± 2.1
Kaempferol diglycoside	37.1	231, 265, 301sh, 346	593	– MS^2 [593]: 285 (100), 284 (5) – MS^3 [593 → 285]: 267 (57), 257 (100), 256 (30), 241 (45), 229 (42), 213 (25), 197 (35), 163 (52) – MS^3 [593 → 284]: 255 (100)	5.0 ± 0.3
Isorhamnetin diglycoside	37.6	230, 255, 264sh, 303sh, 353	623	– MS^2 [623]: 315 (100), 300 (14) – MS^3 [623 → 315]: 300 (100)	1.9 ± 0.2
Isorhamnetin 3-*O*-rutinoside	38.8	233, 255, 264sh, 303sh, 354	623	– MS^2 [623]: 315 (100), 300 (12) – MS^3 [623 → 315]: 300 (100)	112.6 ± 9.6
Isorhamnetin 3-*O*-glucoside	40.0	230, 255, 264sh, 295sh, 354	477	– MS^2 [477]: 315 (100), 300 (11) – MS^3 [477 → 315]: 300 (100)	1.7 ± 0.1

side, based on their UV and mass spectral data and by comparison of the retention times with those of commercially available reference compounds. Another quercetin diglycoside eluting prior to rutin displayed an *m/z* ratio of 609 and UV data exactly matching those of rutin. It is therefore concluded that this compound is a positional isomer of rutin or contains hexoses other than glucose and rhamnose. The fourth flavonol glycoside showed a pseudomolecular ion of *m/z* 593 and a prominent fragment of *m/z* 285 and was therefore identified as a kaempferol with a hexose and a deoxyhexose attached. The detection of flavonol glycosides is in contrast to a recent report by Miean and Mohamed (2001) who screened 62 edible tropical plants for their flavonoid contents and did not find any flavonols in *A. spinosus*.

In extracts of *Boerhavia erecta*, a more complex profile of phenolic compounds was found, although hydroxycinnamates could not be detected. The flavanols were readily identified by comparison with authentic standards except one compound eluting after 19.7 min which could only be characterized as a dimeric procyanidin, based on its UV and MS data.

Among the flavonols, the identical quercetin and kaempferol glycosides were found as in *A. spinosus*, with Q-3-*O*-rutinoside being the predominant compound. In addition, three isorhamnetin (I) glycosides were detected, two of which were unambiguously identified as I-3-*O*-rutinoside and I-3-*O*-glucoside since they matched retention times and UV and MS data of those of reference substances. The third isorhamnetin derivative displayed a pseudomolecular ion of *m/z* 623 and UV data identical to I-3-*O*-rutinoside. It is therefore concluded to be composed of a hexose and a deoxyhexose moiety, too. The assignment of the aglycones to isorhamnetin was based on their fragmentation in the MS^3 event. According to Justesen (2001), methoxylated flavonoid aglycones can be distinguished by mass spectrometry because of their different fragmentation profiles. While the formation of an A-ring fragment of *m/z* 165 as the most prominent ion is a peculiarity of rhamnetin, isorhamnetin glycosides produce an intense fragment of *m/z* 300 in the MS^3 event (Schieber *et al.*, 2002, 2003). In the present study, all of these compounds showed *m/z* 300 fragments in the MS^3 event and were therefore identified as isorhamnetin glycosides.

Betalains

The betalains are responsible for the red appearance of members belonging to the Amaranthaceae and Nyctaginaceae, such as *A. spinosus* and *B. erecta*. Stem bark extracts from the abovementioned plants were compared both with respect to their pigment patterns as well as to their colour qualities. The individual betalains and their relative proportions in the extracts are given in Table II. Betanin and isobetanin from red beet were taken as retention time standards. In *A. spinosus* extracts, two pigments eluted earlier than betanin and isobetanin, indicating a higher degree of glycosylation. The absorption maxima and the MS data ($[M+H]^+$ = 727; $[M+H]^+$ = 551 = 727 – glucuronic acid = betanin; $[M+H]^+$ = 389 = 551 – glucose) pointed to amaranthine (**1**) and its C_{15}-epimer isoamaranthine (**1′**) (Fig. 1; Table II), respectively (Huang and von Elbe, 1986; Cai *et al.*, 2001), amounting to about 95% of the betacyanin fraction. The remainder was betanin (**2**) and isobetanin (**2′**) as proven by retention times, absorption characteristics and mass spectral analyses ($[M+H]^+$ = 551; $[M+H]^+$ = 389 = 551 – glucose) of standard substances from red beet (Fig. 1; Table II). Identical betacyanins at comparable levels have been reported by Cai *et al.* (2001) in *A. spinosus* seeds, however, at a lower epimerization ratio. Another minor compound **5** hitherto not detected in *A. spinosus* could not be assigned more specifically by mass spectrometric data, but showed similar retention and absorption characteristics as **5** in *B. erecta*. While the red pigments of *Amaranthus* sp. are well characterized (Cai *et al.*, 2001), there are only three reports on the occurrence of beta-

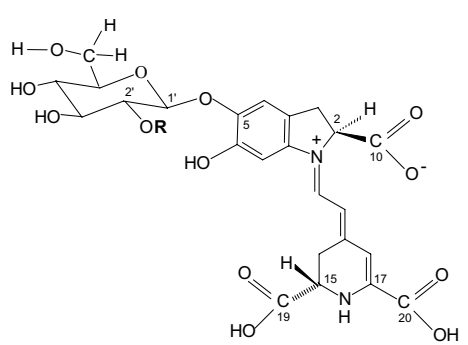

Fig. 1. Structure of betanin (**2**) and amaranthine (**1**).

Table II. Characteristic data and contents of betacyanins from *Amaranthus spinosus* and *Boerhavia erecta.*

	Retention time [min]	HPLC-DAD λ_{max} [nm]	$[M+H]^+$ *m/z*	HPLC-ESI(+)-MS^2 experiment *m/z* (% base peak)	Area at 538 nm (%)	Pigment content [mg/100 g]
Amaranthus spinosus						23.9 ± 0.0[a]
1 Amaranthine	9.6	538	727	MS^2 [727]: 551(28), 389 (100)	63.4	15.13
1′ Isoamaranthine	10.8	538	727	MS^2 [727]: 551(28), 389 (100)	24.6	5.87
2 Betanin	12.2	538	551	MS^2 [551]: 389 (100)	7.4	1.77
2′ Isobetanin	14.3	538	551	MS^2 [551]: 389 (100)	2.1	0.50
5 –[d]	15.6	538	–[e]	–[e]	2.5	0.60
Boerhavia erecta						185.5 ± 0.1[b]
2 Betanin	12.5	538	551	MS^2 [551]: 389 (100)	30.3	56.21
3 –[c]	13.2	505	507	MS^2 [507]: 345 (100)	1.1	2.04
2′ Isobetanin	14.0	538	551	MS^2 [551]: 389 (100)	30.2	56.02
4 –[c]	15.3	505	507	MS^2 [507]: 345 (100)	1.3	2.41
5 –[d]	15.6	538	–[e]	–[e]	2.6	4.82
6 –[d]	17.4	–[e]	–[e]	–[e]	2.0	3.71
7 Neobetanin	18.0	473	549	MS^2 [549]: 387 (100)	30.3[f]	56.21
8 –[d]	28.2	–[e]	–[e]	–[e]	1.1	2.04
9 –[d]	29.3	–[e]	–[e]	–[e]	1.1	2.04

[a] Betacyanin content expressed as amaranthine at 538 nm.
[b] Betalain content expressed as betanin at 538 nm and as neobetanin at 476 nm.
[c] Decarboxylated betanin-derived structure.
[d] Unknown betacyanin structure.
[e] Unambiguous absorption or mass signal could not be obtained.
[f] Area at 476 nm (%).

lains in *Boerhavia* sp., namely in the flowers of *B. coccinea* Mill. (Taylor, 1940), whole plant extracts of *B. erecta*, *B. intermedia* and *B. spicata* (Mabry *et al.*, 1963) and in the leaves of *B. diffusa* L. (Lee and Collins, 2001), however, without further specification. LC-DAD and LC-MS analyses of the *B. erecta* extract revealed that betanin (**2**) and isobetanin (**2′**) were the predominant compounds at virtually equal concentrations (Fig. 1, Table II), accompanied by minute amounts of less polar compounds **3**, **4**, **5** two of which showed identical mass spectra ($[M+H]^+ = 507$; $[M+H]^+ = 345 = 507 - \text{glucose}$), and corresponding absorption maxima (Table II). The mass difference of 44 (551–507) suggested decarboxylated betanin-derived structures. The fact that compounds **3** and **4** displayed identical absorption maxima of 505 nm and similar ratios compared to the betanin-isobetanin pair, indicated that assignment to C_{15}-isomers is plausible. Since the hypsochromic shift of 33 nm can be explained by a reduction of the π-electron delocalization in betanin, the site of decarboxylation is expected to be at C_{17}. This observation complies with a report of Minale *et al.* (1965). Interestingly, in previous studies, decarboxylated structures were mainly ascribed to thermal degradation (Dunkelblum *et al.*, 1972; Schwartz and von Elbe, 1983), with the exception of 2-descarboxybetanin (Kobayashi *et al.*, 2001) being an endogenous pigment from yellow hairy root cultures. All these structures displayed similar or corresponding absorption maxima when compared to betanin because the π-electron system remained unaffected. Since no additional information through mass spectral fragmentation could be obtained and pigments were present in very low amounts, we were not able to conduct further studies on **3** and **4**. However, based on the abovementioned observations, structures **3** and **4** are assumed to be betanin structures decarboxylated at C_{17}. The occurrence of the corresponding aglycones has so far not been reported. Whether these compounds are endogenously synthesized or rather the result of the drying process cannot be answered with certainty at this moment. Other minor betacyanin pigments **6**, **8**, **9** were detected but could not be characterized in more detail (Table II). In contrast, neobetanin (**7**), 14,15-dehydrobetanin, having a yellowish appearance (λ = 473 nm) was identified by both its late retention time as compared to betanin and mass spectral data analyses ($[M+H]^+ = 549$; $[M+H]^+ = 387 =$

549 – glucose). Additional evidence for neobetanin was obtained by the absence of the corresponding C_{15}-epimer, a characteristic of betacyanin structures with an asymmetric carbon at position 15. Neobetanin has rarely been reported as a genuine pigment and was identified in red beet (Alard *et al.*, 1985; Kujala *et al.*, 2001) and prickly pear (Strack *et al.*, 1987). To rule out that neobetanin was an artefact generated under acidic conditions as mentioned by Wyler (1986), betalain extracts were analysed by LC-DAD directly after extraction before removal of phenolics for LC-MS studies. Since up to now thermal treatments are not hold responsible for neobetanin generation at plant physiological pH (Stintzing and Carle, 2004), it is very likely that neobetanin is endogenous to *B. erecta*. This is underlined by the fact that in equally treated *A. spinosus* stem bark extracts with virtually the same pH (5.2 for *B. erecta* and 5.4 for *A. spinosus*) no neobetanin could be detected. The presence of neobetanin was also reflected in the yellower tonality of *B. erecta* ($L^* = 62.59$; $h° = 16.72$; $C^* = 52.80$) compared to *A. spinosus* crude extracts ($L^* = 60.96$; $h° = 5.62$; $C^* = 39.59$). Besides the differing pigment patterns and appearance, *B. erecta* also showed higher betacyanin levels (185.5 mg/100 g) than *A. spinosus* (23.9 mg/100 g). To the best of our knowledge, this is the first report on the qualitative and quantitative pigment pattern within the genus *Boerhavia*.

Phenolics are well-known for their diverse physiological properties including among others anticarcinogenic, antiatherogenic, anti-inflammatory effects that are often subsumed as antioxidant activities (*e.g.,* Di Carlo *et al.*, 1999; Ma and Kinneer, 2002; Middleton *et al.*, 2000; Rice-Evans *et al.*, 1997; Vinson *et al.*, 1998, 2001). Similar properties have only very recently been suggested for the betalains (Cai *et al.*, 2003; Kanner *et al.*, 2001; Tesoriere *et al.*, 2003; Wettasinghe *et al.*, 2002). Thus, it seems likely that these compounds may partly contribute to the pharmacological effects of their traditional applications. Finally, the obtained data present a valuable contribution for the scientific evaluation of pharmacologically active principles in *A. spinosus* and *B. erecta*.

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