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.

\textit{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/
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 sea-sand 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 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 thermosto sampler model G1329/1330A, a column oven model G1316A, and a diode array detector model G1315A. The column used was an Aqua 5 µm C18 (250 × 4.6 mm I.D.) from Phenomenex (Torrance, CA, USA) and a security guard C18 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 (hydroxy-cinnamic 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 C18(2)
reversed phase column with a particle size of 5 µm (Phenomenex, Torrance, CA, USA), fitted with a security guard C18 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 ($\varepsilon = 60000 \text{l/mol} \cdot \text{cm}$; $\lambda = 538 \text{nm}$; molecular weight = 550; Wyler and Meuer, 1979) and neobetanin ($\varepsilon = 18200 \text{l/mol} \cdot \text{cm}$; $\lambda = 476 \text{nm}$; molecular weight = 548; Wyler and Meuer, 1979) for Boerhavia erecta and of amaranthine ($\varepsilon = 56600 \text{l/mol} \cdot \text{cm}$; $\lambda = 538 \text{nm}$; 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 ≤ $A \leq 0.95$ at $\lambda_{\text{max}}$. L*ab* 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 D65 and 10° observer angle, metric chroma ($C^* = (a^*2 + b^*2)^{1/2}$) and hue angle ($h^\circ = \arctan (b^*/a^*)$) were obtained by the transformation of a* and b* cartesian coordinates into polar ones according to $C^* = (a^*2 + b^*2)^{1/2}$ and $h^\circ = \arctan (b^*/a^*)$.

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 (MS³) 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 A. spinosus and B. 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 MS² and MS³ 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-cafeoylquinic 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 mucro-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-gluc-
<table>
<thead>
<tr>
<th>Phenolics from <em>Amaranthus spinosus</em> and <em>Boerhavia erecta</em></th>
<th>Retention time [min]</th>
<th>HPLC-DAD λ&lt;sub&gt;max&lt;/sub&gt; [nm]</th>
<th>M–H&lt;sup&gt;−&lt;/sup&gt; m/z</th>
<th>HPLC-ESI(-)-MS&lt;sup&gt;n&lt;/sup&gt; experiment</th>
<th>Content [mg/100 g]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amaranthus spinosus</strong></td>
<td></td>
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<tr>
<td>Caffeoylquinic acid</td>
<td>15.6</td>
<td>243, 302sh, 327</td>
<td>353</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [353]: 191 (62), 173 (100), 155 (4), 111 (15)</td>
<td>109.2 ± 15.6</td>
</tr>
<tr>
<td>Caffeoylquinic acid</td>
<td>16.1</td>
<td>234, 314</td>
<td>353</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [353]: 191 (60), 173 (100), 155 (7), 111 (18)</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Coumaroylquinic acid</td>
<td>21.3</td>
<td>233, 301sh, 314</td>
<td>337</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [337]: 173 (100), 155 (4), 111 (18)</td>
<td>54.6 ± 6.0</td>
</tr>
<tr>
<td>Coumaroylquinic acid</td>
<td>22.6</td>
<td>232, 310</td>
<td>337</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [337]: 173 (100), 155 (5), 111 (19)</td>
<td>17.5 ± 2.0</td>
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<tr>
<td>Feruloylquinic acid</td>
<td>24.2</td>
<td>239, 302sh, 328</td>
<td>367</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [367]: 173 (100), 155 (10), 111 (31)</td>
<td>57.4 ± 5.5</td>
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<tr>
<td>Feruloylquinic acid</td>
<td>25.0</td>
<td>234, 322</td>
<td>367</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [367]: 173 (100), 155 (8), 111 (25)</td>
<td>6.5 ± 0.2</td>
</tr>
<tr>
<td>Quercetin diglycoside</td>
<td>25.9</td>
<td>231, 257, 264sh, 300sh, 357</td>
<td>609</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [609]: 301 (100), 300 (37)</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Quercetin 3-O-rutinoside</td>
<td>26.6</td>
<td>231, 256, 264sh, 302sh, 354</td>
<td>609</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [609]: 301 (100), 300 (21)</td>
<td>36.4 ± 9.8</td>
</tr>
<tr>
<td>Quercetin 3-O-glucoside</td>
<td>28.0</td>
<td>231, 256, 263sh, 302sh, 354</td>
<td>463</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [463]: 301 (100), 300 (15)</td>
<td>9.0 ± 1.9</td>
</tr>
<tr>
<td>Kaempferol diglycoside</td>
<td>33.5</td>
<td>231, 265, 300sh, 348</td>
<td>593</td>
<td>– MS&lt;sup&gt;2&lt;/sup&gt;; [593]: 285 (100), 284 (6)</td>
<td>7.0 ± 1.8</td>
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<tr>
<td><strong>Boerhavia erecta</strong></td>
<td></td>
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<tr>
<td>Procyanidin B1</td>
<td>10.4</td>
<td>233, 279</td>
<td>577</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [577]: 559 (31), 451 (30), 425 (100), 407 (71), 289 (21)</td>
<td>7.8 ± 0.5</td>
</tr>
<tr>
<td>Catechin</td>
<td>12.9</td>
<td>233, 279</td>
<td>289</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [289]: 245 (100), 205 (33), 203 (14), 179 (14)</td>
<td>19.5 ± 0.2</td>
</tr>
<tr>
<td>Procyanidin B2</td>
<td>14.6</td>
<td>232, 280</td>
<td>577</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [577]: 559 (28), 451 (40), 425 (100), 407 (58), 289 (17)</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>17.3</td>
<td>232, 280</td>
<td>289</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [289]: 245 (100), 205 (30), 203 (14), 179 (17)</td>
<td>7.0 ± 0.5</td>
</tr>
<tr>
<td>Dimeric procyanidin</td>
<td>19.7</td>
<td>232, 279</td>
<td>577</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [577]: 559 (19), 451 (14), 425 (100), 407 (75), 289 (10)</td>
<td>19.2 ± 0.7</td>
</tr>
<tr>
<td>Quercetin diglycoside</td>
<td>29.6</td>
<td>230, 255, 264sh, 298sh, 354</td>
<td>609</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [609]: 301 (100), 300 (54)</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>Quercetin 3-O-rutinoside</td>
<td>30.3</td>
<td>234, 256, 263sh, 301sh, 353</td>
<td>609</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [609]: 301 (100), 300 (27)</td>
<td>133.4 ± 8.1</td>
</tr>
<tr>
<td>Quercetin 3-O-glucoside</td>
<td>31.8</td>
<td>231, 256, 263sh, 303sh, 354</td>
<td>463</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [463]: 301 (100), 300 (16)</td>
<td>10.8 ± 2.1</td>
</tr>
<tr>
<td>Kaempferol diglycoside</td>
<td>37.1</td>
<td>231, 265, 301sh, 346</td>
<td>593</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [593]: 285 (100), 284 (5)</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>Isorhamnetin diglycoside</td>
<td>37.6</td>
<td>230, 255, 264sh, 303sh, 354</td>
<td>623</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [623]: 315 (100), 300 (14)</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Isorhamnetin 3-O-rutinoside</td>
<td>38.8</td>
<td>233, 255, 264sh, 303sh, 354</td>
<td>623</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [623]: 315 (100), 300 (12)</td>
<td>112.6 ± 9.6</td>
</tr>
<tr>
<td>Isorhamnetin 3-O-glucoside</td>
<td>40.0</td>
<td>230, 255, 264sh, 295sh, 354</td>
<td>477</td>
<td>– MS&lt;sup&gt;3&lt;/sup&gt;; [477]: 315 (100), 300 (11)</td>
<td>1.7 ± 0.1</td>
</tr>
</tbody>
</table>
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 detected 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 MS3 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 MS3 event (Schieber et al., 2002, 2003). In the present study, all of these compounds showed m/z 300 fragments in the MS3 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 above-mentioned 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 C15-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).](image-url)
Table II. Characteristic data and contents of betacyanins from *Amaranthus spinosus* and *Boerhavia erecta*.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Retention time [min]</th>
<th>HPLC-DAD λ&lt;sub&gt;max&lt;/sub&gt; [nm]</th>
<th>[M+H]&lt;sup&gt;+&lt;/sup&gt;</th>
<th>HPLC-ESI(+)-MS&lt;sub&gt;2&lt;/sub&gt; m/z (% base peak)</th>
<th>Area at 538 nm (%)</th>
<th>Pigment content [mg/100 g]</th>
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<tbody>
<tr>
<td><strong>Amaranthus spinosus</strong></td>
<td></td>
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<tr>
<td>1 Amaranthine</td>
<td>9.6</td>
<td>538</td>
<td>727</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [727]: 551(28), 389 (100)</td>
<td>63.4</td>
<td>23.9 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1&lt;sup&gt;′&lt;/sup&gt; Isoamaranthine</td>
<td>10.8</td>
<td>538</td>
<td>727</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [727]: 551(28), 389 (100)</td>
<td>24.6</td>
<td>15.13</td>
</tr>
<tr>
<td>2 Betanin</td>
<td>12.2</td>
<td>538</td>
<td>551</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [551]: 389 (100)</td>
<td>7.4</td>
<td>5.87</td>
</tr>
<tr>
<td>2&lt;sup&gt;′&lt;/sup&gt; Isobetanin</td>
<td>14.3</td>
<td>538</td>
<td>551</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [551]: 389 (100)</td>
<td>2.1</td>
<td>0.50</td>
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<tr>
<td>5&lt;sup&gt;−d&lt;/sup&gt;</td>
<td>15.6</td>
<td>538</td>
<td>551</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [551]: 389 (100)</td>
<td>2.5</td>
<td>0.60</td>
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<tr>
<td><strong>Boerhavia erecta</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>185.5 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 Betanin</td>
<td>12.5</td>
<td>538</td>
<td>551</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [551]: 389 (100)</td>
<td>30.3</td>
<td>56.21</td>
</tr>
<tr>
<td>3&lt;sup&gt;−e&lt;/sup&gt;</td>
<td>13.2</td>
<td>505</td>
<td>507</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [507]: 345 (100)</td>
<td>1.1</td>
<td>2.04</td>
</tr>
<tr>
<td>4&lt;sup&gt;−c&lt;/sup&gt;</td>
<td>15.3</td>
<td>505</td>
<td>507</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [507]: 345 (100)</td>
<td>1.3</td>
<td>2.41</td>
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<tr>
<td>5&lt;sup&gt;−d&lt;/sup&gt;</td>
<td>15.6</td>
<td>538</td>
<td>507</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [507]: 345 (100)</td>
<td>2.6</td>
<td>4.82</td>
</tr>
<tr>
<td>6&lt;sup&gt;−d&lt;/sup&gt;</td>
<td>17.4</td>
<td>538</td>
<td>507</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [507]: 345 (100)</td>
<td>2.0</td>
<td>3.71</td>
</tr>
<tr>
<td>7 Neobetanin</td>
<td>18.0</td>
<td>473</td>
<td>549</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [549]: 387 (100)</td>
<td>30.3&lt;sup&gt;f&lt;/sup&gt;</td>
<td>56.21</td>
</tr>
<tr>
<td>8&lt;sup&gt;−d&lt;/sup&gt;</td>
<td>28.2</td>
<td>473</td>
<td>549</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [549]: 387 (100)</td>
<td>1.1</td>
<td>2.04</td>
</tr>
<tr>
<td>9&lt;sup&gt;−d&lt;/sup&gt;</td>
<td>29.3</td>
<td>473</td>
<td>549</td>
<td>MS&lt;sup&gt;2&lt;/sup&gt; [549]: 387 (100)</td>
<td>1.1</td>
<td>2.04</td>
</tr>
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</table>

<sup>a</sup> Betacyanin content expressed as amaranthine at 538 nm.
<sup>b</sup> Betalain content expressed as betanin at 538 nm and as neobetanin at 476 nm.
<sup>c</sup> Decarboxylated betanin-derived structure.
<sup>d</sup> Unknown betacyanin structure.
<sup>e</sup> Unambiguous absorption or mass signal could not be obtained.
<sup>f</sup> 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<sup>′</sup>) 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]<sup>+</sup> = 507; [M+H]<sup>+</sup> = 345 = 507 – 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<sub>15</sub>-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<sub>17</sub>. 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-decarboxybetanin (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 above-mentioned observations, structures 3 and 4 are assumed to be betanin structures decarboxylated at C<sub>17</sub>. 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]<sup>+</sup> = 549; [M+H]<sup>+</sup> = 387 =
Additional evidence for neo-
betanin was obtained by the absence of the corre-
sponding C15-epimer, a characteristic of betacya-
nin 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 held 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 re-
lected in the yellower tonality of B. erecta (L* =
62.59; h* = 16.72; C* = 52.80) compared to A. spi-
nosus crude extracts (L* = 60.96; h* = 5.62; C* =
39.59). Besides the differing pigment patterns and
appearance, B. erecta also showed higher betacya-
nin 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 quantita-
tive pigment pattern within the genus Boerhavia.

Phenolics are well-known for their diverse phys-
iological properties including among others anti-
carcinogenic, antiatherogenic, anti-inflammatory
effects that are often subsumed as antioxidant
activities (e.g., Di Carlo et al., 1999; Ma and Kin-
neer, 2002; Middleton et al., 2000; Rice-Evans
et al., 1997; Vinson et al., 1998, 2001). Similar prop-
erties 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 scien-
tific evaluation of pharmacologically active prin-
ciples in A. spinosus and B. erecta.

Acknowledgements

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