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Identification of Proanthocyanidin Dimers and Trimers, Flavone *C*-Glycosides, and Antioxidants in *Ficus deltoidea*, a Malaysian Herbal Tea

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ABSTRACT: Phenolic compounds in an aqueous infusion of leaves of *Ficus deltoidea* (Moraceae), a well-known herbal tea in Malaysia, were analyzed by HPLC coupled to photodiode array and fluorescence detectors and an electrospray ionization tandem mass spectrometer. Following chromatography of extracts on a reversed phase C_{12} column, 25 flavonoids were characterized and/or tentatively identified with the main constituents being flavan-3-ol monomers, proanthocyanidins, and *C*-linked flavone glycosides. The proanthocyanidins were dimers and trimers comprising (epi)catechin and (epi)afzelechin units. No higher molecular weight proanthocyanidin polymers were detected. The antioxidant activity of *F. deltoidea* extract was analyzed using HPLC with online antioxidant detection. This revealed that 85% of the total antioxidant activity of the aqueous *F. deltoidea* infusion was attributable to the flavan-3-ol monomers and the proanthocyanidins.

KEYWORDS: *Ficus deltoidea*, flavan-3-ol monomers, dimers and trimers, flavone *C*-glycosides, HPLC-PDA-MS^2, antioxidants

INTRODUCTION

Ficus deltoidea (Moraceae) is a shrub commonly known as Mas cotek or Serapat angin in Malaysia, Tabat barito in Indonesia, and Kangkalibang in Africa. It is has been reported that *F. deltoidea*, consumed principally as a tea prepared by aqueous infusion of the leaves, possesses various medicinal effects including aphrodisiac activity and antihypertension, antidiabetic, and anticancer properties.[1] An aqueous extract of *F. deltoidea* fed to rats exhibited antidiabetic properties with no side effects.[2] Phytochemical studies on a wide number of *Ficus* spp., including *F. carica*, *F. indica*, and *F. racemosa*, have revealed that phenolics are major components.[3,4] *Ficus* spp. are also reported to have antioxidant activity that has been attributed to the presence of flavonoids as well as terpenoids, alkaloids,[5] and tannins.[6] However, little is known about the actual phytochemical content, and, in particular, the flavonoid composition of *F. deltoidea*.

Flavonoids are $C_6-C_3-C_6$ compounds that in recent years have attracted an enormous amount of interest due to their potential protective effects on human health. They have antioxidant, anticarcinogenic, anti-inflammatory, antiviral, hepatoprotective, and antiallergenic properties.[7,8] Flavan-3-ols are a subclass of flavonoids that can form oligomeric and polymeric proanthocyanidins, which are also known as condensed tannins. Proanthocyanidins consisting of (−)-epicatechin/(+)-catechin units are called procyanidins and are the most abundant type of proanthocyanidins in plants. The less common proanthocyanidins containing (−)-epiafzelechin/(+)afzelechin and (−)epigallocatechin/(+)-gallocatechin subunits are called propelargonidins and prodelphinidins, respectively.[9] Proanthocyanidins have been reported to be anticancer agents and to have antiatherosclerotic effects.[10−12] Flavones are another subgroup of flavonoids that are found in many herbs, typically as *O*- or *C*-linked glycosides. They are reported to have antioxidant, anti-inflammatory, antimutagenic, anticancer, and anti-HIV activities.[13]

There is growing interest in the phytochemical content and potential beneficial effects of *F. deltoidea* consumption, which has gained increasing popularity among the general population in Malaysia. The dried leaves, which are widely available in local markets, are made into a tea. In addition to being served as a tea, *F. deltoidea* extracts are also sold commercially in capsule form as an herbal supplement. In the present study, HPLC-MS^2-based methodology was used to analyze the flavonoid compounds in aqueous extracts of *F. deltoidea*. In addition, to identify specific compounds with antioxidant activity extracts were analyzed by HPLC with an online antioxidant detection system.[14]

MATERIALS AND METHODS

Chemicals and Reagents. HPLC grade methanol was purchased from Rathburn (Walkerburn, U.K.). The flavonoid standards, (+)-catechin, apigenin-8-*C*-glucoside (vitexin), apigenin-6-*C*-glucoside (isovitexin), and luteolin-6-*C*-glucoside (isoorientin), were obtained from Extrasynthese (Genay, France). Gallic acid and delphinidin-3-galactoside were supplied by AASC Ltd. (Southampton, U.K.). Procyanidin B_2, (−)-epicatechin, formic acid, phosphoric acid, metaphosphoric acid, Folin−Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were purchased from Sigma-Aldrich (Dorset, U.K.). Other chemicals were supplied by Fisher Scientific (Leicestershire, U.K.).

Plant Material. Dried leaves of *F. deltoidea* purchased from a local market in Pahang, Malaysia, were dried further for 2 days at 45 °C before

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being ground to a powder, which was extracted with boiling water for 1 h, after which the infusion was filtered and the filtrate spray-dried to form a powder, which was shipped to Glasgow by courier in an airtight plastic container.

Sample Preparation. A 100 mg powdered *F. deltoidea* extract was dissolved in 10 mL of methanol/water (1:1, v/v) and centrifuged at 10000*g* for 5 min at 4 °C prior to analysis.

Determination of Total Phenol Content. The total phenolic of *F. deltoidea* extract was measured in gallic acid equivalents using Folin−Ciocalteu according to the method described by Singleton and Rossi.[15]

Ferric Reducing Antioxidant Potential (FRAP) Assay. The antioxidant activity of *F. deltoidea* extracts was estimated using the FRAP assay.[16] The antioxidant activity was expressed as the mean concentration of Fe^{2+} produced per millimole.

HPLC-PDA-MS^2 with Online Antioxidant Detection. Analyses were carried out using a Surveyor HPLC system (Thermo Scientific, Waltham, MA), comprising a pumping system, an autosampler, and a degasser coupled to a photodiode array absorbance (PDA) detector scanning from 200 to 700 nm controlled by Xcalibur software version 1.3. Separations were carried out using a MAX-RP 4 μm, 250 mm × 4.6 mm, C_{12} reversed phase column (Phenomenex, Torrance, CA) maintained at 40 °C and eluted at a flow rate of 1.0 mL/min with a 60 min gradient from 15 to 50% methanol in water containing 0.1% formic acid. After passing through the flow cell of the PDA detector column, eluate was directed to a fluorescence detector (Jasco FP-920 with excitation at 280 nm and emission at 315 nm), then split, and 20% was directed to an LCQ Duo mass spectrometer (Thermo Scientific) with an electrospray interface operating with negative ionization in full scan data dependent MS^2 mode from 150 to 1000 amu. For the detection of components with antioxidant activity, the remaining 800 μL/min of the HPLC eluate was mixed with an ABTS solution flowing at 0.5 mL/min and the resultant mixture passed through a holding coil before being directed to a P2000 absorbance detector (Thermo Scientific) operating at 720 nm.[14] Data were analyzed using Xcalibur software, and peaks were quantified in Trolox equivalents.

Analysis of Procyanidins. Thiolytic degradation was carried out on a dried sample reacted with 400 μL of benzyl mercaptan (5% in methanol, v/v) in 200 μL of acidified methanol (3.3% HCl, v/v) at 40 °C for 30 min, with samples being vortexed every 10 min.[17] HPLC-PDA-FL-MS^2 analysis was performed with conditions as described above except for the use of a 60 min gradient of 3−55% acetonitrile in 1% aqueous formic acid.

RESULTS

Identification of Phenolic Compounds. Three aqueous extracts of *F. deltoidea* were analyzed in triplicate by gradient reversed phase HPLC with PDA, fluorescence, antioxidant, and MS detection. HPLC fluorescence and absorbance profiles at 280 and 365 nm are presented in Figure 1. The mass spectral properties of the 26 numbered peaks along with their identification/partial identification are summarized in Table 1. The identifications were based on the following criteria:

Peak 1 (t_R, 6.6 min; λ_{max}, 270 nm) was gallocatechin based on cochromatography with a (+)-gallocatechin and a negatively charged molecular ion $[M - H]^-$ at m/z 305, which yielded MS^2 fragments at m/z 261, 221, and 179. Reversed phase HPLC does not separate the (+)- and (−)-gallocatechins, so the enantiomer is not specified. This is also the case with the other flavan-3-ols that were detected.

Peak 2 (t_R, 11.4 min; λ_{max}, 275 nm) was identified as epigallocatechin as it coeluted with a standard and both had a $[M - H]^-$ at m/z 305 and MS^2 ions at m/z 261, 221, and 179.

Peak 3 (t_R, 12.5 min; λ_{max}, 280) was catechin based on cochromatography with a standard. Both compounds were fluorescent

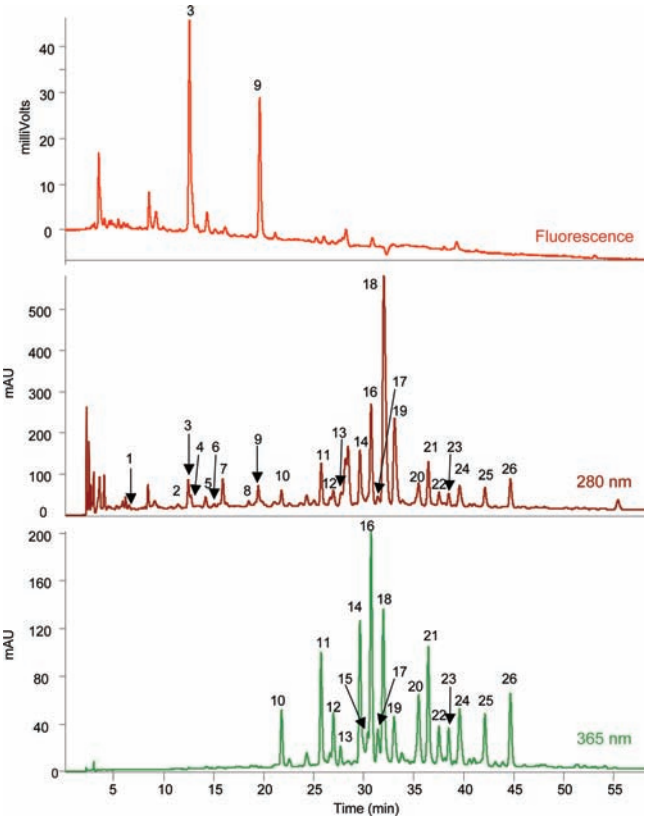

Figure 1. Reversed phase HPLC-MS of an aqueous infusion of *F. deltoidea* leaves with detection by fluorescence and absorbance at 280 and 365 nm. For MS^2 identification of numbered peaks, see Table 1.

and had a $[M - H]^-$ at m/z 289, which fragmented to produce daughter ions at m/z 245, 205, and 179.

Peaks 4, 5, and 7 (t_R, 12.7, 14.2, and 15.9 min; λ_{max}, 280 nm) were all identified as type-B dimers of (epi)catechin and (epi)afzelechin. This identification was made on the basis of each yielding a $[M - H]^-$ at m/z 561, which upon MS^2 produced ions at m/z 435 as well as at m/z 425 [retro-Diels−Alder reaction (RDA)], m/z 407, m/z 289 (an (epi)catechin unit), and m/z 271 (an (epi)afzelechin unit) as reported by De Souza et al.[18] The m/z 407 results from water elimination of m/z 425. The heterocyclic ring fission of the dimer produces m/z 435 (loss of 126 Da). The quinone methide cleavage of the interflavan bond gives rise to m/z 271 and 289, which respectively indicate an (epi)afzelechin extension unit and an (epi)catechin terminal unit as reported by Verardo et al.[19]

Peaks 6 and 8 (t_R, 15.1 and 18.5 min; λ_{max}, 275 nm) both had the properties of type B procyanidin trimer with a $[M - H]^-$ ion at m/z 833, and the resulting MS^2 spectrum composed of ions at m/z 561 ($[M - H - 272]^-$), m/z 543 ($[M - H - 290]^-$), m/z 289, and m/z 271. The MS^2 fragments at m/z 271 and 289 again indicate that (epi)afzelechin is located in the upper unit, whereas (epi)catechin constitutes the lower terminal unit. MS^2 also produced minor fragment ions at m/z 697 and 679, which are in keeping with RDA fission. MS^3 led to fragment ions at m/z 425 and 407, RDA reaction of a dimer. Both peaks therefore yield spectra indicative of the presence of a type B proanthocyanidin trimer consisting of two (epi)afzelechin units with an (epi)catechin terminal unit, in keeping with the findings of De Souza et al.[18]

Table 1. Mass Spectral Characteristics and Identity of Phenolics Present in an Aqueous Infusion of *Ficus deltoidea* Leaves Analyzed by HPLC-MS^{2a}

peak	t_R (min)	λ_{max}	compound	$[M - H]^-$ (m/z)	MS^2 fragments ions (m/z)
1	6.6	270	gallocatechin	305	261, 221, 179
2	11.4	275	epigallocatechin	305	261, 221, 179
3	12.5	280	catechin	289	245, 205, 179
4	12.7	280	(epi)afzelechin-(epi)catechin	561	435, 425, 407, 289, 271
5	14.2	280	(epi)afzelechin-(epi)catechin	561	435, 425, 407, 289, 271
6	15.1	275	(epi)afzelechin-(epi)afzelechin-(epi)catechin	833	561, 543, 289, 271
7	15.9	280	(epi)afzelechin-(epi)catechin	561	435, 425, 407, 289, 271
8	18.5	275	(epi)afzelechin-(epi)afzelechin-(epi)catechin	833	561, 543, 289, 271
9	19.4	280	epicatechin	289	245, 205, 179
10	21.8	350	luteolin-6,8-*C*-diglucoside (lucenin-2)	609	519, 489, 399
11	25.7	340	apigenin-6,8-*C*-diglucoside (vicenin-2)	593	503, 473, 353
12	27.0	345	luteolin-6-*C*-hexosyl-8-*C*-pentoside	579	489, 459, 399
13	27.8	345	luteolin-6-*C*-glucosyl-8-*C*-arabinoside	579	489, 459, 399
14	29.6	335	apigenin-6-*C*-arabinosyl-8-*C*-glucoside (isoschaftoside)	563	503, 473, 443
15	30.4	345	luteolin-6-*C*-arabinosyl-8-*C*-glucoside	579	489, 459, 399
16	30.7	335	apigenin-6-*C*-glucoside-8-*C*-arabinoside (schaftoside)	563	503, 473, 443
17	31.4	335	luteolin-8-*C*-glucoside (orientin)	447	369, 357, 327
18	31.9	320	apigenin-6-*C*-pentosyl-8-*C*-glucoside	563	473, 443, 353
19	33.0	310	4-*p*-coumarolyquinic acid	337	191, 173, 163
20	35.5	335	apigenin-8-*C*-glucoside (vitexin)	431	413, 341, 311
21	36.5	335	apigenin-6-*C*-glucosyl-8-*C*-pentoside	563	473, 443, 353
22	37.5	335	apigenin-6,8-*C*-dipentoside isomer	533	515, 473, 443
23	38.5	335	apigenin-6,8-*C*-dipentoside isomer	533	515, 473, 443
24	39.6	335	apigenin-6-*C*-glucoside (isovitexin)	431	413, 341, 311
25	42.1	335	apigenin-6,8-*C*-dipentoside isomer	533	515, 473, 443
26	44.7	335	apigenin-6,8-*C*-dipentoside isomer	533	515, 473, 443

[a] Peak numbers and retention times refer to Figure 1. t_R, retention time, $[M - H]^-$, negatively charged molecular ion.

Peak 9 (t_R, 19.4 min; λ_{max}, 280 nm) also produced a strong fluorescent peak and was identified as epicatechin based on a $[M - H]^-$ at m/z 289, which gave rise to MS^2 ions at m/z 245, 205, and 179.The identification was confirmed by cochromatography with an authentic standard.

Peak 10 (t_R, 21.8 min; λ_{max}, 350 nm) had a $[M - H]^-$ at m/z 609, and its MS^2 spectrum consisted of ions at m/z 489 ($[M - H - 120]^-$), m/z 519 ($[M - H - 90]^-$), and m/z 399 ($[M - H - 120 - 90]^-$), corresponding to the fragmentation of a flavone *C*-diglycoside, which, based on the data of Ferreres et al.,[20] is tentatively identified as luteolin-6,8-*C*-diglucoside (also known as lucenin-2).

Peak 11 (t_R, 25.7 min; λ_{max}, 340 nm) was characterized by a $[M - H]^-$ at m/z 593, which produced daughter ions at m/z 503 ($[M - H - 90]^-$) and m/z 473 ($[M - H - 120]^-$), indicative of the cleavage of a *C*-glycoside. These fragments, based on the findings of Han et al.,[21] are in keeping with the presence of apigenin-6,8-*C*-diglucoside (also known as vicenin-2).

Peak 12 (t_R, 27.0 min; λ_{max}, 345 nm) exhibited a $[M - H]^-$ ion at m/z 579 and MS^2 fragments at m/z 489 ($[M - H - 90]^-$) and m/z 459 ($[M - H - 120]^-$), in keeping with the presence of a *C*-linked hexose sugar, m/z 399 ($[M - H - 120 - 60]^-$), corresponding to the further fragmentation of a pentose unit, and m/z 285. These data indicate the presence of luteolin (m/z 285) + hexose (m/z 162) + pentose (m/z 132). Thus, peak 12, in keeping with the data of Figueirinha et al.,[22] is tentatively identified as either a luteolin-6-*C*-hexosyl-8-*C*-pentoside or a luteolin-8-*C*-hexosyl-6-*C*-pentoside.

Peak 13 (t_R, 27.8 min; λ_{max}, 345 nm) and peak 15 (t_R, 30.4 min; λ_{max}, 345 nm) both had $[M - H]^-$ ions at m/z 579 and an MS^2 spectrum with ions at m/z 489 ($[M - H - 90]^-$), m/z 459 ($[M - H - 120]^-$), indicating the presence of a *C*-linked hexose moiety, and m/z 399 ($[M - H - 180]^-$). This is a *C*-glycosylflavone fragmentation pattern similar to that reported by Ferreres et al.,[23] and on the basis of their elution sequence peak 13 is tentatively identified as luteolin-6-*C*-glucosyl-8-*C*-arabinoside and peak 15 as luteolin-6-*C*-arabinosyl-8-*C*-glucoside.

Peak 14 (t_R, 29.6 min; λ_{max}, 335 nm) and peak 16 (t_R, 30.7 min; λ_{max}, 335 nm) both had a $[M - H]^-$ at m/z 563, which produced daughter ions at m/z 503 ($[M - H - 60]^-$), m/z 473 ($[M - H - 90]^-$), and m/z 443 ($[M - H - 120]^-$). The ($[M - H - 60]^-$) indicates a pentose substitution, in addition to that of a hexose sugar. By reference to the mass spectrometric and chromatographic data of Han et al.,[21] peak 14 was tentatively identified as apigenin-6-*C*-arabinosyl-8-*C*-glucoside (also known as isoschaftoside) and peak 16 as apigenin-6-*C*-glucosyl-8-*C*-arabinoside (also known as schaftoside).

Peak 17 (t_R, 31.4 min; λ_{max}, 335 nm) had a $[M - H]^-$ at m/z 447 and an MS^2 spectra with ions at m/z 357 ($[M - H - 90]^-$) and m/z 327 ($[M - H - 120]^-$). In keeping with the findings of Kazuno et al.[24] and cochromatography with a reference compound, this peak was identified as luteolin-8-*C*-glucoside (also known as orientin).

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Peak 18 (t_R, 31.9 min; λ_{max}, 320 nm) and peak 21(t_R, 36.5 min; λ_{max}, 335 nm) gave the same $[M - H]^-$ ion at m/z 563, and its MS^2 spectrum consisted of ions at m/z 473 ($[M - H - 90]^-$), the base peak, and m/z 443 ($[M - H - 120]^-$). On examination of the fragment ions, it was observed that their relative abundances were similar to those reported by Ferreres et al.,[20] suggesting that peak 18 was apigenin-6-*C*-arabinosyl-8-*C*-glucoside. The fragmentation pattern of peak 21 resembles data reported by Han et al.,[21] suggesting that the peak may be apigenin-6-*C*-glucosyl-8-*C*-pentoside.

Peak 19 (t_R, 33.0 min; λ_{max}, 310 nm) was a chlorogenic acid with a $[M - H]^-$ at m/z 337 and MS^2 fragments at m/z 191, 173, and 163. On the basis of the fragmentation patterns reported by Clifford et al.,[25] this peak is 4-*p*-coumaroylquinic acid.

Peak 20 (t_R, 35.5. min; λ_{max}, 335 nm) and peak 24 (t_R, 9.6 min; λ_{max}, 335 nm) both had an $[M - H]^-$ ion at m/z 431 and MS^2 ions at m/z 341 ($[M - H - 90]^-$) and m/z 311 ($[M - H - 120]^-$). Cochromatography and the mass spectrometric properties of reference compounds identified peak 20 as apigenin-8-*C*-glucoside (also known as vitexin) and peak 24 as apigenin-6-*C*-glucoside (also known as isovitexin).

Peak 22 (t_R, 37.5 min; λ_{max}, 335 nm), peak 23 (t_R, 38.5 min; λ_{max}, 335 nm), peak 25 (t_R, 42.1 min; λ_{max}, 335 nm), and peak 26 (t_R, 44.7 min; λ_{max}, 335 nm) all had a $[M - H]^-$ at m/z 533, which yielded MS^2 fragments at m/z 443 ($[M - H - 90]^-$) and m/z 311 ($[M - H - 60]^-$), which are likely to be due to the presence of a pentose substitution. There was no $[M - H - 120]^-$ fragment, indicating the absence of a *C*-hexose conjugate. The fragmentation suggests apigenin (271) + pentose (132) + pentose (132). Peaks 22, 23, 25, and 26 are therefore tentatively identified as apigenin-6,8-*C*-dipentosides with likely pentose sugars being arabinose and xylose.

Analysis of Proanthocyanidins. No polymeric flavan-3-ols were detected by analysis after thiolytic degradation, as no adducts were formed during the reaction and the catechin/epicatechin peaks did not increase significantly.

Total Phenols and Antioxidant Activity. The total phenolic content of the aqueous infusion from leaves of *F. deltoidea* was 6.3 ± 0.2 mmol/L of gallic acid equivalents, whereas FRAP antioxidant activity was 7.2 ± 0.2 mmol/L of Fe^{2+}. These values are high, equivalent to those obtained with purple grape juice and cloudy apple juice by Mullen et al.[26]

HPLC data obtained with the online ABTS antioxidant detection system are illustrated in Figure 2 along with absorbance traces at 280 and 365 nm. The chromatographic profiles are illustrated from 6 to 34 min as none of the later eluting peaks exhibited antioxidant activity. The peaks contributing the main antioxidant activity were the flavan-3-ol monomers gallocatechin (peak 1), catechin (peak 3), and epicatechin (peak 9) and the flavone apigenin-6,8-*C*-diglucoside (peak 11), and the data on the contribution of individual compounds the overall ABTS antioxidant activity are summarized in Table 2. The flavan-3-ols were the main antioxidants, contributing 85%, compared to the 15.0% for the flavones that was due principally to apigenin-6,8-*C*-diglucoside.

DISCUSSION

A total of 25 flavonoids (9 flavan-3-ols and 16 flavones) and 1 chlorogenic acid, 4-*p*-coumaroylquinic acid, were identified or tentatively identified in *F. deltoidea* with proanthocyanidins and flavones as major compounds detected (Tables 1 and 2). Other *p*-coumaroylquinic acid isomers were not detected when the total ion current chromatogram was scanned for the molecular ion at

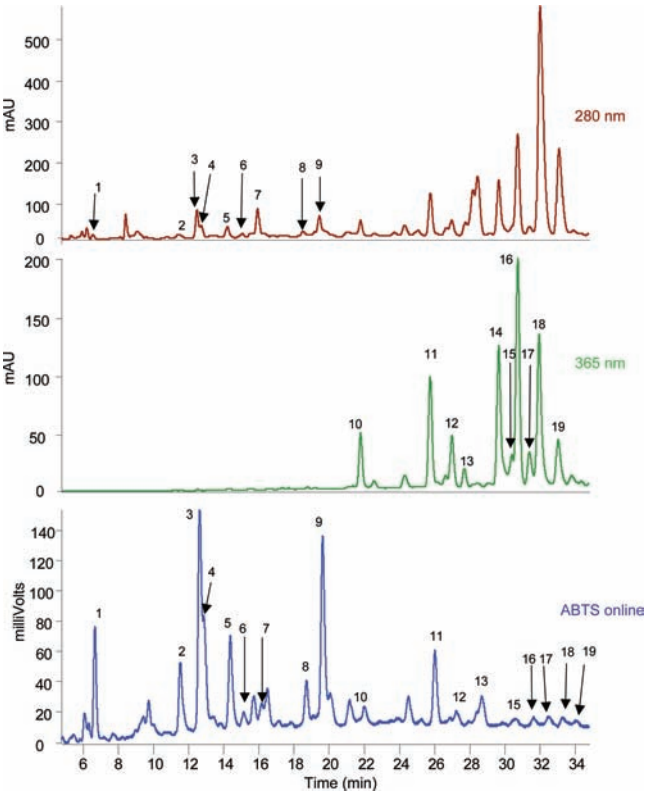

Figure 2. Reversed phase HPLC of an aqueous infusion of *F. deltoidea* leaves with absorbance detection at 280 and 365 nm and online $ABTS^+$ antioxidant detection at 720 nm. For MS^2 identification of numbered peaks see Table 1.

m/z 337. Such compounds, if present, would be in very small amounts, which is unusual because plants producing chlorogenic acids generally contain several subgroups or isomers.[27] It is notable that the dominance of 4-*p*-coumaroylquinic acid relative to other compounds in *Ficus* spp. has not been reported previously in other species. Thus, a *F. deltoidea* infusion is a convenient source for 4-*p*-coumaroylquinic acid.

Four flavan-3-ol monomers were detected, namely, catechin, epicatechin, gallocatechin, and epigallocatechin, which also occur in green tea prepared from young leaves of *Camellia sinensis*.[28,29] Five oligomers of proanthocyanidins were also detected, consisting, unusually, of (epi)catechin and (epi)afzelechin subunits. The main fragmentation pathways for these proanthocyanidins are RDA fission and subsequent elimination of water, heterocylic ring fission, and interflavanic bond cleavage through the quinone–methine mechanism.[30] Further analysis of the *F. deltoidea* extract after thiolytic degradation did not detect the presence of additional proanthocyanidin with a degree of polymerization of >3.

Sixteen flavone *C*-glycosides were detected. Luteolin and apigenin derivatives were the main flavones present in *F. deltoidea*, and among them were isomers of schaftoside and vitexin (Table 2), which have not been previously reported to occur in other *Ficus* species.[31,32] The differences in the flavonoid content of *F. deltoidea* and other fig species may be related to genuine interspecies differences or geographical and/or environmental effects.[33] The high level of phenolics contents in *F. deltoidea* is similar to that reported in other *Ficus* spp.,[34] although the main phenolics may vary from one species to another.[31] Because *F. deltoidea* is typically consumed as an herbal tea, it is worth noting that the high level of

Table 2. Content and Antioxidant Potential of Individual Compounds of Aqueous Infusion of *Ficus deltoidea* Leaves[a]

compound (peak)	concentration (μmol/L)	Trolox equivalent (μmol/L)	antioxidant activity (%)
flavan-3-ols			
gallocatechin (1)	44 ± 1	78 ± 3	9.4
epigallocatechin (2)	87 ± 2	68 ± 2	8.2
catechin (3)	98 ± 1	197 ± 4	24
(epi)afzelechin-(epi)catechin (4)	18 ± 0	69 ± 1	8.3
(epi)afzelechin-(epi)catechin (5)	21 ± 0	87 ± 1	11
(epi)afzelechin-(epi)afzelechin-(epi)catechin (6)	5.7 ± 0	11 ± 0	1.4
(epi)afzelechin-(epi)catechin (7)	39 ± 0	7.2 ± 0	0.9
(epi)afzelechin-(epi)afzelechin-(epi)catechin (8)	5.1 ± 0	32 ± 1	3.9
epicatechin (9)	89 ± 3	148 ± 3	18
total	**407**	**697**	**85.1**
flavones			
luteolin-6,8-*C*-diglucoside (lucenin-2) (10)	13 ± 0	15 ± 1	1.8
apigenin-6,8-*C*-diglucoside (vicenin-2) (11)	15 ± 1	76 ± 1	9.1
luteolin-6-*C*-hexosyl-8-*C*-pentoside (12)	11 ± 0	0	0
luteolin-6-*C*-glucosyl-8-*C*-arabinoside (13)	3.9 ± 0	0	0
apigenin-6-*C*-arabinosyl-8-*C*-glucoside (isoschaftoside) (14)	7.4 ± 0	0	0
luteolin-6-*C*-arabinosyl-8-*C*-glucoside (15)	2.0 ± 0	9.5 ± 0	1.1
apigenin-6-*C*-glucosyl-8-*C*-arabinoside (schaftoside) (16)	33 ± 0	13 ± 1	1.5
luteolin-8-*C*-glucoside (orientin) (17)	10 ± 0	9.0 ± 0	1.1
apigenin-6-*C*-pentosyl-8-*C*-glucoside (18)	117 ± 0	3.6 ± 0	0.4
apigenin-8-*C*-glucoside (vitexin) (20)	7.8 ± 0	0	0
apigenin-6-*C*-glucosyl-8-*C*-pentoside (21)	7.0 ± 0	0	0
apigenin-6,8-*C*-dipentoside isomer (22)	2.2 ± 0	0	0
apigenin-6,8-*C*-dipentoside isomer (22)	1.1 ± 0	0	0
apigenin-6-*C*-glucoside (Isovitexin) (23)	15 ± 0	0	0
apigenin-6,8-*C*-dipentoside isomer (24)	9.3 ± 0	0	0
apigenin-6,8-*C*-dipentoside isomer (24)	14 ± 0	0	0
total	**269**	**126**	**15.0**
hydroxycinnamates			
4-*p*-coumaroylquinic acid (19)	139 ± 3	3.3 ± 0	0.4
total	**813**	**826**	**100**

[a] Results represent concentration of phenolics and antioxidants in an aqueous infusion of *F. deltoidea* leaves (10 g/L). For information on peak numbers see Table 1 and Figure 2.

total phenolics is comparable with those occurring in black and green teas[35] as well as fruit juices.[26]

The *F. deltoidea* tea exhibited both a high total phenol content and antioxidant activity. HPLC with online detection of antioxidant activity demonstrated that the flavan-3-ol monomers and the (epi)catechin-(epi)afzelechin dimers and trimers accounted for 85% of the antioxidant activity of the aqueous infusion of *F. deltoidea* leaves. Proanthocyanidins, which are widely distributed in pine bark, berries, grapes, and cocoa products,[9] have become a focus of interest due to their radical-scavenging properties, anti-inflammatory activity, and anticarcinogenic effects.[36−39]

Flavones, which are active components in many herbs, have been found to affect human health. Apigenin possesses pharmacological effects with its antioxidant, antiviral, anti-inflammatory, and anticancer properties,[40,41] whereas luteolin has anti-inflammatory effects.[42] Both apigenin and luteolin are reported to have protective effects against vascular diseases[43] and are also potential inhibitors of cellular autoimmunity.[44] As mentioned earlier, *F. deltoidea* has antidiabetic properties,[2] and arguably this could at least in part be due to the presence of *C*-glycosylflavonoids, such as vitexin, isovitexin, and orientin. Owing to *C*-3′-hydroxylation of the B ring of flavones, these *C*-glycosylflavones possess strong α-glucosidase inhibitory activity, higher than that of acarbose, a drug used for treating type 2 diabetes mellitus.[45]

In conclusion, these findings suggest that the HPLC-PDA-MS^2 analytical method has been successful in discriminating a total of 26 phenolics, with flavan-3-ol monomers, proanthocyanidins, a diversity of flavone *C*-glycosides, and the exclusive occurrence of a single chlorogenic acid in an aqueous infusion of *F. deltoidea* leaves. The chromatographic fingerprinting obtained is also useful for the herbal standardization of *F. deltoidea* herbal products. Most of the antioxidant activity of the infusion was attributed to the flavan-3-ol monomers and proanthocyanidins.

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