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Profiling the chemical content of *Ficus lyrata* extracts *via* UPLC-PDA-qTOF-MS and chemometrics

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This study attempts to elucidate the secondary metabolite profiles of *Ficus lyrata* leaves and fruits grown in Egypt. Non-targeted metabolite profiling *via* ultra performance liquid chromatography (UPLC)-qTOF-MS was used to identify various chemical classes in *F. lyrata* fruits and leaves (i.e. flavonoids, phenolic acids and fatty acids) analysed by chemometrics. A total of 72 metabolites were evaluated *via* a UPLC-qTOF-MS-based metabolomic study. Seventeen flavonoids were characterised and tentatively identified with the main constituents being catechins/procyanidins, *O*- and *C*-linked flavonoid glycosides. The major procyanidins were dimers and trimers comprising (epi)catechin and (epi)afzelechin units, whereas the predominant flavones were *C*-glycosides of luteolin and apigenin. Aside from these major flavonoid classes, a group of benzoic acids, caffeoylquinic acids, fatty acid and sphingolipids were also annotated. This study provides the most complete map for polyphenol distribution in *F. lyrata* leaves and fruits and the basis for future investigation of its fruits nutritional value or possible nutraceutical uses.

Keywords: *Ficus lyrata*; metabolite profiling; UPLC/MS; flavonoids; fatty acids; PCA

1. Introduction

Ficus, the fig genus, consists of *ca.* 800 species of woody trees and shrubs in the family Moraceae (Lansky et al. 2008). The most widespread species in the genus is *Ficus carica*, which produces the commercial fruits called fig (Slavin 2006; Veberic et al. 2008). Other notable species of ficus are *Ficus religiosa* L., *Ficus elastica* Roxb., *Ficus benghalensis* L., *Ficus racemosa* L. and *Ficus benjamina* L. (Shiksharthi & Mittal 2011; Da Cruz et al. 2012; Kumar et al. 2012). *Ficus lyrata* is a less commonly seen member of the *Ficus* family and commonly referred as 'fiddle leaf fig'. The tree is grown singly as a shade tree, or in groups along roads and in some botanical gardens in Egypt (Loutfy et al. 2005; Lansky et al. 2008). The strong antioxidant activity of *F. lyrata* L. growing in Egypt (Abdel-Hameed 2009) prompted the investigation of its chemical composition. In addition, only scant phytochemical studies are reported in the literature for this species (Basudan et al. 2005).

The current progress in plant metabolomic techniques has made it possible to detect several hundred natural compounds and to compare samples reliably for differences and similarities in a semi-automated and untargeted manner (Bedair & Sumner 2008; Schripsema 2010; Sumner & Hall 2013). A recent technique includes the use of ultra-performance liquid chromatography (UPLC) which in comparison with conventional LC achieves rapid metabolite analysis and can

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obtain better peak separation than usually possible with standard LC methods. UPLC coupled with high-resolution qTOF-MS is a relatively new technology that can detect chemical compounds with high sensitivity (Grata et al. 2009). The use of UPLC-qTOF-MS for quality control assessment of phytomedicines and commercial plant drug preparations has been reported for the analyses of green tea, ginseng, artichoke and hypericum (Farag & Wessjohann 2012; Farag et al. 2013). The objective of this research is to define and compare *F. lyrata* fruits and leaves, grown in Egypt, targeting both its secondary and primary metabolites *via* UPLC-PDA-ESI-TOF-MS^n. Multivariate data analyses, e.g. principal component analysis (PCA), were employed to ensure good analytical rigorousness and define both similarities and differences among both organs. In this study, the structure of the identified compounds from *F. lyrata* leaves and fruits are presented in Figure 1.

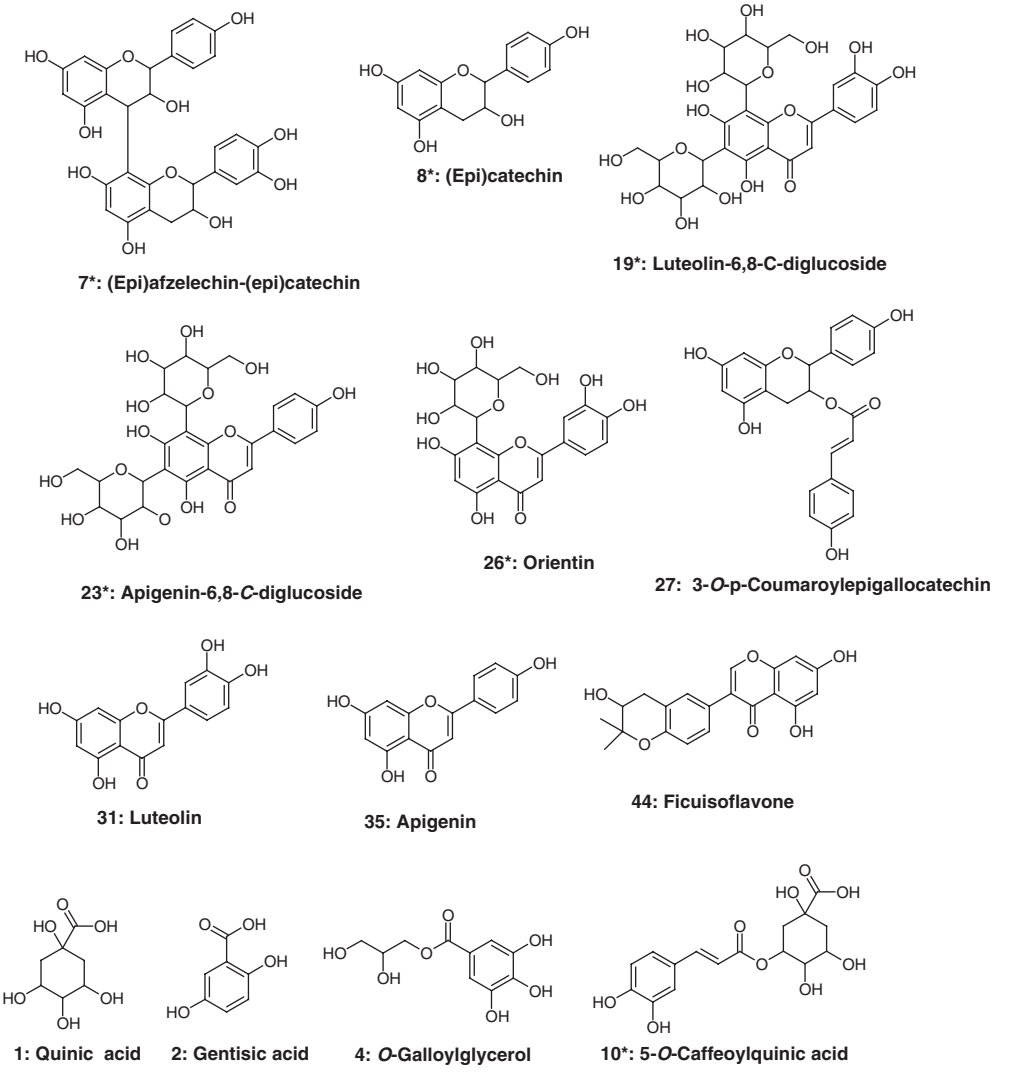

Figure 1. Proposed constitution of flavonoids (A) and phenolic acids derivatives (B) from *F. lyrata* leaves and fruits identified by using UPLC-PDA-qTOF-MS (Table S1).

2. Results and discussion

2.1 *Identification of metabolites in* F. lyrata *fruits and leaves* via *UPLC/PDA/MS*

To assess the metabolite composition in *F. lyrata*, a non-targeted metabolite profiling of extracts derived from fruits and leaves was conducted. Methanol-soluble constituents were analysed by a high-resolution UPLC/PDA/(−)ESI-qTOF-MS, using a gradient mobile phase of acetonitrile and aqueous acetic acid that allowed for a comprehensive elution of *Ficus* analytes (Figure S1). A total of 72 metabolites were detected. The identities, retention times, UV characteristics, and observed molecular and fragment ions for individual components are presented in Table S1. Identified metabolites belonged to various classes including phenolic acid conjugates (i.e. *O*-caffeoyl quinic acid and gentisic acid), flavonoids (i.e. apigenin and luteolin *C*-glycosides), catechin/procyanidins (i.e. epiafzelechin–epicatechin), sphingolipids and fatty acids (i.e. hydroxy-octadecatrienoic acid and hydroxy-octadecadienoic acid), with flavonoids and phenolic acids as the most abundant classes in both organs.

2.1.1 *Identification of flavonoids*

Flavonoids have been reported from different species of *Ficus* (Sheu et al. 2005; Veberic et al. 2008). Several flavonoid peaks found in leaf samples show intense molecular ion peaks with M-90 and M-120 fragments in MS^n spectrum indicative of the cleavage of a *C*-glycoside. Previous results demonstrated that luteolin and apigenin glycosides present in *Ficus* were of the *C*-glycoside type (Omar et al. 2011). Peak 23 was characterised by an $[M - H]^-$ at *m/z* 593 with fragment ions at *m/z* 503 ($[M - H - 90]^-$) and *m/z* 473 ($[M - H - 120]^-$) in accordance with the presence of apigenin-6,8-*C*-diglucoside (vicenin-2) (Han et al. 2008). Peak 19 had an $[M - H]^-$ at *m/z* 609, and its MS^n spectrum show ions at *m/z* 489 ($[M - H - 120]^-$), *m/z* 519 ($[M - H - 90]^-$), corresponding to the fragmentation of a flavone *C*-diglycoside, tentatively identified as luteolin-6,8-*C*-diglucoside (i.e. as lucenin-2) (Ferreres et al. 2003). Other *C*-glycosides identified include orientin in peak 26 $[M - H]^-$ at *m/z* 447 and an MS^n spectra with ions at *m/z* 357 ($[M - H - 90]^-$) and *m/z* 327 ($[M - H - 120]^-$) and matching with authentic standard. It should be noted that several methylated flavones were detected in fruits peaks (39, 42 and 46), albeit not in leaves which suggests an increased activity of one or more *O*-methyltransferases in fruits relative to leaves, and with all peaks showing the characteristic loss of methyl from its methoxy group (− 15 Da).

UV spectra of compounds from peaks 25 and 28 suggested that they are flavonol-*O*-glycosides. In MS/MS analysis, the nature of the sugar residues could be revealed from their loss or cleavage daughter peaks, that is, 162 amu (hexose; glucose or galactose) or 146 amu (rhamnose). In detail, rutin (peak 25) was characterised by an $[M - H]^-$ at *m/z* 609.146, with a main fragment ion at *m/z* 301.0429 ($C_{16}H_{11}O_7$, aglycon ion) derived from the loss of a hexose and rhamnose moieties. The MS/MS spectrum of compound **28** shows an $[M - H]^-$ at *m/z* 463.132, with a main fragment ion at *m/z* 301.0429 ($C_{16}H_{11}O_7$, aglycon ion) identified as isoquercetin, derived from the loss of a hexose moiety. Notably, *O*-glycosides were not identified in luteolin/apigenin peaks suggestive for the presence of an *O*-glycosyl transferase enzyme with a substrate specificity that excludes flavones. The versatility and exquisite substrate and site specificity of flavonoid-modifying enzymes is an ongoing topic of investigation (Luo et al. 2007).

A-type proanthocyanidins flavanone dimers are widely distributed in several plant families. In this study, (epi)catechin and (epi)afzelechin (7) were identified in *F. lyrata* as type-B dimer. In addition, novel dimer (12) and trimer (17) of (epi)gallocatechin and (epi)afzelechin were found in *F. lyrata*. In detail, identification of (epi)catechin and (epi)afzelechin ($[M - H]^-$, *m/z* 561) in peak 7 was based on an MS fragment at *m/z* 435 and 425 [retro-Diels–Alder (RDA) reaction], *m/z* 289 (an (epi)catechin unit) and *m/z* 271 (an (epi)afzelechin unit) (De Souza et al. 2008).

Cleavage of the interflavan bond gives rise to *m/z* 271 and 289 which indicate an (epi) afzelechin and an (epi)catechin unit, respectively (Figure S2). Similarly, peak 17 had the properties of a type B procyanidin trimer with an $[M - H]^-$ ion at *m/z* 849, and the resulting MS^n spectrum composed of ions at *m/z* 577 $[M - H - 272]^-$, *m/z* 559 $[M - H - 290]^-$, *m/z* 289 and *m/z* 271. The MS^n fragments at *m/z* 271 and 289 again indicate that epiafzelechin is located in the upper unit, whereas (epi)catechin constitutes the lower terminal unit. MS^n led to fragment ions at *m/z* 441 and 423, RDA reaction of a dimer. This is the first report of an (epi) gallocatechin type B procyanidin dimer and trimers in *Ficus* species. A novel (epi)catechin di-galloyl rhamnoside was identified in peak (6) showing catechin-like RDA fragmentation $[M - H - (2 \times 152)]^-$ with MS^n ions appearing at *m/z* 587 as well as at *m/z* 435, indicative of the presence of two galloyl moieties with λ_{max} at 278 nm (Wu et al. 2003). The *m/z* 289 for (epi) catechin results from one further loss of a rhamnose moiety (− 146 amu), see Figure S3.

2.1.2 *Identification of phenolic acids*

Derivatives formed from the interaction of hydroxycinnamic acids with quinic acid are common phenolics in many plant families contributing to its taste (Pink et al. 1994). The predominant fragment of 191 amu for quinic acid in the MS^n spectrum of peaks (9–11) and characteristic UV_{max} values at 298 and 325 nm are diagnostic for *O*-caffeoyl quinic acids (Clifford et al. 2006). Other phenolic acids identified in this study include *O*-*p*-coumarolyquinic acid (15), gentisic acid (2), its glycoside (5) and *O*-galloyl glycerol (4), all evident from their characteristic UV_{max} and mass spectral data (Table S1).

2.1.3 *Identification of fatty acids and sphingolipids*

In the second half of the chromatographic run (400–700 s), MS spectra of several unsaturated fatty acids, i.e. oleic (72), linoleic (69) and linolenic acid (68) were readily identified, as evident from high-resolution masses at *m/z* 281.2486, 279.2335 and 277.2174 with predicted molecular formulae of $C_{18}H_{33}O_2^-$, $C_{18}H_{31}O_2^-$ and $C_{18}H_{29}O_2^-$, respectively. These were the least polar metabolites of all chromatograms and thus eluted with the highest acetonitrile percentile at the end of the runs. Some MS signals were also assigned to saturated fatty acids, i.e. specifically palmitic acid (70) as evident from high-resolution mass at *m/z* 255.2316 with predicted molecular formulae of $C_{16}H_{31}O_2^-$. Negative ionisation MS also revealed several hydroxy fatty acids being responsible for the major peaks 36, 38, 56 and 62. In detail, peaks 36 and 38 provide a mass weight of 327.2173 and *m/z* 329.2333 amu, with predicted molecular formulas of $C_{18}H_{31}O_5^-$ and $C_{18}H_{33}O_5^-$, respectively, and a loss of two water molecules (18 amu), suggestive of extra hydroxy groups. A mass difference of 2 amu between (36) and (38) is indicative of an extra double bond and are tentatively identified as trihydroxy octadecadienoic acid and trihydroxy octadecenoic acids (oxylipids). Several peaks, i.e. 52 and 54, show an even mass weight of 474.2621 and 476.2779 amu with predicted molecular formulas of $C_{23}H_{41}NO_7P^-$ and $C_{23}H_{43}NO_7P^-$, respectively, in the negative ionisation mode (Table S1). Most cellular sphingolipids are ionised by electrospray and can be detected in negative ESI modes (Shui et al. 2010). Inspection of tandem MS data also reveals the loss of *m/z* 285 indicative of a sphingosine moiety in peak 57 (Scherer et al. 2010). The detailed analysis and full structural elucidation to determine oxygenation position in oxygenated fatty acids is still under way.

2.2 *Multivariate PCA analysis of UPLC-MS data*

PCA is an unsupervised clustering method requiring no knowledge of the data-set and acts to reduce the dimensionality of multivariate data while preserving most of the variance (Goodacre

et al. 2000). Triplicate biological measurements from the same sample were found to be highly reproducible, as the scores of replicate measurements were more or less superimposed (Figure S4). Considering 72 variables as analytical data, PCA was able to discriminate among varieties. Three principal components (PCs) were required to capture almost 99% of the variance. The main PC to differentiate between samples, i.e. PC1, accounted for 93% of the variance with the fruits samples located to the left of the vertical line representing PC1 (negative PC1 values) whereas leaf samples were positioned to the right of PC1 (Figure S4A). The segregation observed in PCA score plot can be explained in terms of the identified compounds, using the loading plots for PC1 signals that expose those peaks (compounds) that have the largest effect on the respective principle component. As revealed in Figure S4B, the major group that stood out in this plot corresponded to the MS signals of caffeoyl quinic acids (9–11) and fatty acid conjugates (69, 70 and 72) which contribute negatively to PC1 in addition to a methylated apigenin aglycone (39). In addition, the MS signals for catechin (8) and hydroxy fatty acids (56 and 62) contributed positively to PC1. These results point to a divergent fatty acid metabolism in *Ficus* organs with polar fatty acids being more enriched, in general, in leaves versus fruits. The effect of caffeic acid conjugates, fatty acids and methylated flavones were also apparent from the comparative UPLC-UV-MS representation in Figure S1 (peaks denoted with an asterisk) and absolute quantification of phenolics in Table 1. As can be seen from Table 1, 5-*O*-caffeoylquinic acid was the predominant phenolic compound of fruits and leaves, with fruits showing the highest levels (35.7 mg/g, peak 10). The levels of 5-*O*-caffeoylquinic acid in fruits amounted to more than to twofold compared to leaves (16.7 mg/g), whereas levels of (epi) catechin (19) and epiafzelechin–(epi)catechin (12) in leaves amounted to *ca.* 34 and 10 times its constitution in fruit. *C*-flavonoid glycosides (peaks 19, 23 and 24) were almost found exclusively in leaves at a level of 0.3–0.6 mg/g and absent in fruits. 5-*O*-caffeoylquinic acid is one of the major phenolic acids in many *Ficus* species including *F. carica* L. (Oliveira et al. 2009). In addition, the proanthocyanidins with epicatechin and epiafzelechin units and *C*-flavonoid glycosides were previously identified in *Ficus deltoidea* (Omar et al. 2011).

3. Experimental

3.1 *Plant material and extraction procedure*

Fresh fruits and leaves of *F. lyrata* were collected from 6-October Garden, Helmeyet Elzaiton, Cairo, Egypt (2012) and authenticated by Dr Emad Saleh, Phytochemistry and Plant Systematics, Desert Research Center, Cairo, Egypt. Voucher specimens of *F. lyrata* fruits (MA-01-100) and leaves (MA-01-101) have been deposited at the Chemistry Department, Faculty of Science, Helwan University, Cairo, Egypt. The freeze-dried fruits and leaves were ground separately in a mortar with liquid nitrogen. The powder (150 mg) was then homogenised with 6 mL 100% MeOH containing 5 μg/mL umbelliferone (internal standard) using a Turrax mixer

Table 1. Quantification of metabolites identified in *F. lyrata* fruits and leaves using MS detection.

Metabolites	Fruits	Leaves	Fruits/leaves
5-*O*-caffeoylquinic acid	16.75 ± 0.39	35.79 ± 0.97	0.47
Epicatechin	14.83 ± 0.05	0.44 ± 0.04	33.7
Epiafzelechin–epicatechin	0.692 ± 0.08	0.07 ± 0.0 3	9.9
Luteolin-6,8-*C*-diglucoside	0.394 ± 0.12	Traces	–
Apigenin-6,8-*C*-diglucoside	0.628 ± 0.17	Traces	–
Luteolin-*C*-hexosyl-pentoside	0.295 ± 0.07	Traces	–
Orientin	0.03 ± 0.01	Traces	–

Note: Values are expressed as mg g^{-1} for three biological replicates ± SD.

(IKA-Werke GmbH, Staufen, Germany) (11,000 rpm) for five 20 s periods. Extracts were then vortexed and centrifuged at 3000 rpm for 30 min to remove plant debris. For LC–MS analyses, 500 μL were aliquoted, stored at − 20°C till it was used for injection.

3.2 *High-resolution UPLC-PDA-MS analysis*

Chromatographic separation was performed on an Acquity UPLC system (Waters, En Yvelines Cedex, France) coupled to an online PDA and MS detector following the exact procedure described earlier (Farag et al. 2012). Quantification of the glycosylated derivatives of luteolin, epicatechins and phenolic acids was calculated from the calibration curve of luteolin, epicatechin and chlorogenic acid standards, respectively, using a PDA detector. Calibration curves from standards were obtained for each reference compound using concentrations spanning from 0.1, 1, 10 and 100 $\mu g\,mL^{-1}$. Assays were carried out in triplicate.

3.3 *UPLC-MS data processing and multivariate analysis*

Relative quantification of metabolite profiles after UPLC-MS was performed using XCMS data analysis software, which can be downloaded freely as an R package from the Metlin Metabolite Database (http://137.131.20.83/download/) (Smith et al. 2006).

4. Conclusions

To our knowledge, this study provides the most complete map for polyphenol composition in *F. lyrata* grown in Egypt using untargeted large-scale metabolite analysis. The abundance of a catechin monomer pool in leaves, as revealed from PCA analysis, is likely to account for the enrichment of its downstream dimer and trimer type-B procyanidins (Table S1). Together (epi) afzelechin–(epi)gallocatechin and (epi)afzelechin–(epi)catechin are 30 and 40 times more abundant in leaves than in fruits, respectively. Antioxidant activity assays of the aqueous infusion of *F. deltoidea* leaves demonstrated that the flavan-3-ol monomers, dimers and trimers accounted for 85% of its antioxidant activity. A similar scenario could be found in *F. lyrata* leaves and may account for its marked antioxidant activity compared to other *Ficus* species (Abdel-Hameed 2009). Further isolation and *in vitro* testing of metabolites will help provide more evidence for the possible use of *F. lyrata* (crude extract, fractions, sub-fractions or pure compounds) for the prevention or therapy of diseases in which oxidants or free radicals are involved.

Supplementary material

Supplementary material relating to this article is available online, alongside Table S1 and Figures S1–S4 are available online.

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References

Abdel-Hameed ESS. 2009. Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. Food Chem. 114:1271–1277.

Basudan OM, Ilyas M, Parveen M, Muhisen HM, Kumar R. 2005. A new chromone from *Ficus lyrata*. J Asian Nat Prod Res. 7:81–85.

Bedair M, Sumner LW. 2008. Current and emerging mass-spectrometry technologies for metabolomics. TrAC Trends Anal Chem. 27:238–250.

Clifford MN, Knight S, Surucu B, Kuhnert N. 2006. Characterization by LC-MS^n of four new classes of chlorogenic acids in green coffee beans: dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, and feruloyl-dimethoxycinnamoylquinic acids. J Agric Food Chem. 54:1957–1969.

Da Cruz RC, Agertt V, Boligon AA, Janovik V, Anraku de Campos MM, Guillaume D, Athayde ML. 2012. *In vitro* antimycobacterial activity and HPLC-DAD screening of phenolics from *Ficus benjamina* L. and *Ficus luschnathiana* (Miq.) Miq. leaves. Nat Prod Res. 26:2251–2254.

De Souza LM, Cipriani TR, Iacomini M, Gorin PAJ, Sassaki GL. 2008. HPLC/ESI-MS and NMR analysis of flavonoids and tannins in bioactive extract from leaves of *Maytenus ilicifolia*. J Pharm Biomed Anal. 47:59–67.

Farag MA, El-Ahmady SH, Elian FS, Wessjohann LA. 2013. Metabolomics driven analysis of artichoke leaf and its commercial products *via* UPLC-q-TOF-MS and chemometrics. Phytochemistry. 95:177–187.

Farag MA, Porzel A, Wessjohann LA. 2012. Comparative metabolite profiling and fingerprinting of medicinal licorice roots using a multiplex approach of GC-MS, LC-MS and 1D NMR techniques. Phytochemistry. 76:60–72.

Farag MA, Wessjohann LA. 2012. Metabolome classification of commercial *Hypericum perforatum* (St. John's Wort) preparations via UPLC-qTOF-MS and chemometrics. Planta Med. 78:488–496.

Ferreres F, Silva BM, Andrade PB, Seabra RM, Ferreira MA. 2003. Approach to the study of *C*-glycosyl flavones by ion trap HPLCPAD-ESI/MS/MS: application to seeds of quince (*Cydonia oblonga*). Phytochem Anal. 14:352–359.

Goodacre R, Shan B, Gilbert RJ, Timmin EM, McGovern AC, Alsberg BK, Kell DB, Logan NA. 2000. Detection of the dipicolinic acid biomarker in *Bacillus* spores using Curie-point pyrolysis mass spectrometry and fourier transform infrared spectroscopy. Anal Chem. 72:119–127.

Grata E, Guillarme D, Glauser G, Boccard J, Carrupt PA, Veuthey JL, Rudaz S, Wolfender JL. 2009. Metabolite profiling of plant extracts by ultra-high-pressure liquid chromatography at elevated temperature coupled to time-of-flight mass spectrometry. J Chromatogr A. 1216:5660–5668.

Han J, Ye M, Qiao X, Xu M, Wang BR, Guo DA. 2008. Characterization of phenolic compounds in the Chinese herbal drug *Artemisia annua* by liquid chromatography coupled to electrospray ionization mass spectrometry. J Pharm Biomed Anal. 47:516–525.

Kumar A, Rawat S, Tiwari SS, Srivastava A. 2012. Comparative botanical and phytochemical evaluation of medicinally important stem bark of *Ficus* species. Asian Pac J Trop Dis. 2:S33–S37.

Lansky EP, Paavilainen HM, Pawlus AD, Newman RA. 2008. *Ficus* spp. (fig): Ethnobotany and potential as anti cancer and anti inflammatory agents. J Ethnopharmacol. 119:195–213.

Loutfy MHA, Karakish EAK, Khalifa ASF, Mera ERA. 2005. Numerical taxonomic evaluation of leaf architecture of some species of genus *Ficus* L. Int J Agric Biol. 7:352–357.

Luo J, Nishiyama Y, Fuell C, Taguchi G, Elliott K, Hill L, Tanaka Y, Kitayama M, Yamazaki M, Bailey P. 2007. Convergent evolution in the BAHD family of acyl transferases: identification and characterization of anthocyanin acyl transferases from *Arabidopsis thaliana*. Plant J. 50:678–695.

Oliveira AP, Valentão P, Pereira JA, Silva BM, Tavares F, Andrade PB. 2009. *Ficus carica* L.: metabolic and biological screening. Food Chemical Toxicol. 47:2841–2846.

Omar MH, Mullen W, Crozier A. 2011. Identification of proanthocyanidin dimers and trimers, flavone *C*-glycosides, and antioxidants in *Ficus deltoidea*, a Malaysian herbal tea. J Agric Food Chem. 59:1363–1369.

Pink D, Naczk M, Baskin K, Shahidi F. 1994. Theoretical-analysis of ultraviolet–visible spectra of various phenolic-acid fractions of canola. J Agric Food Chem. 42:1317–1322.

Scherer M, Leuthäuser-Jaschinski K, Ecker J, Schmitz G, Liebisch G. 2010. A rapid and quantitative LC-MS/MS method to profile sphingolipids. J Lipid Res. 51:2001–2011.

Schripsema J. 2010. Application of NMR in plant metabolomics: techniques, problems and prospects. Phytochem Anal. 21:14–21.

Sheu YW, Chiang LC, Chen IS, Chen YC, Tsai IL. 2005. Cytotoxic flavonoids and new chromenes from *Ficus formosana*. Planta Med. 71:1165–1177.

Shiksharthi AR, Mittal S. 2011. *Ficus Racemosa:* phytochemistry, traditional uses and pharmacological properties: a review. Int J Recent Adv Pharm Res. 4:6–15.

Shui G, Guan XL, Low CP, Chua GH, Goh JSY, Yang H, Wenk MR. 2010. Toward one step analysis of cellular lipidomes using liquid chromatography coupled with mass spectrometry: application to *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* lipidomics. Mol Biosyst. 6:1008–1017.

Slavin JL. 2006. Figs: past, present and future. Nutr Today. 41:180–184.

Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G. 2006. XCMS: processing mass spectrometry data for metabolite profiling using nonlinear peak alignment, matching, and identification. Anal Chem. 78:779–787.

Sumner LW, Hall RD. 2013. Metabolomics across the globe. Metabolomics. 9:258–264.
Veberic R, Colaric M, Stampar F. 2008. Phenolic acids and flavonoids of fig fruit (*Ficus carica* L.) in the northern Mediterranean region. Food Chem. 106:153–157.
Wu Q, Wang M, Simon JE. 2003. Determination of proanthocyanidins in grape products by liquid chromatography/mass spectrometric detection under low collision energy. Anal Chem. 75:2440–2444.