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# LC-MS$^n$ identification and characterization of the phenolic compounds from the fruits of *Flacourtia indica* (Burm. F.) Merr. and *Flacourtia inermis* Roxb.

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ABSTRACT

The phenolic compounds of the fruits of *Flacourtia indica* (Burm. F.) Merr. and *Flacourtia inermis* (Roxb.), mostly cultivated and consumed in Sri Lanka were investigated qualitatively by HPLC tandem mass spectrometry and high resolution mass spectrometry. Thirty-five phenolic compounds were detected and characterized on the basis of their unique fragmentation pattern in the negative ion mode tandem MS spectra. Twelve of them were extracted for the first time from these sources and four of them were not reported previously in nature. It was also possible to distinguish between the isobaric (same molecular weight) phenolic compounds, dicaffeoylquinic acids and caffeoylquinic acid glycosides. For the positive identification of phenolic compounds by LC-MS$^n$ a series of experiments were carried out. This is the first report for the full characterization of phenolic compounds of the fruits of *F. indica* and *F. inermis* by LC-MS$^n$.



## Introduction

Phenolic compounds are ubiquitous plant metabolites found in fruits, vegetables, grains and beverages relevant to the human diet. The dietary intake of phenolics ranges from 20 mg to 1 g per person per day. These phenolic compounds show a variety of biological activities like anti-oxidant (Idowu et al., 2010), anti-inflammatory (Bayeta & Lau, 2000), anti-HIV (He et al., 1997), anti-HBV (Han, Huang, Yang, Liu, & Wu, 2008) and radical scavenging. They also inhibit mutagenesis and carcinogenesis (Stich, Rosin, & Bryson, 1982) and are considered to be beneficial to human health. On the other hand, these phenolic compounds are involved in UV protection, UV sensing and defense from herbivores and pathogens in plants. Some phenolic compounds have also been reported to provide reproductive advantage as attractants of pollinators and seed dispersers.

Some most common phenolic compounds are hydroxycinnamic acids, hydroxycinnamates, hydroxycinnamic acid glycosides, hydroxybenzoic acids, hydroxybenzoates, hydroxybenzoic acid glycosides, flavonoids and flavonoid glycosides. Classically, chlorogenic acids (CGAs) are a family of esters formed between quinic acid and certain *trans*-hydroxycinnamic acids, most commonly caffeic, *p*-coumaric and ferulic acids (Clifford, 1999, 2000; IUPAC, 1976; Kuhnert, Karakoese, & Jaiswal, 2012); sinapic acid and dimethoxycinnamic acid are also present in certain plant species. Representative structures are shown in Fig. 1.

*Flacourtia indica* (Burm. F.) Merr. and *Flacourtia inermis* Roxb. of the family Flacourtiaceae are moderate size trees growing in Sri Lanka. Fruits of these plants are round, cherry sized, edible and very popular due to their sweet, sour and astringent taste. Ripe fruit of *F. indica* are dark brown and ripe fruit of *F. inermis* are bright pink colored. *F. indica* and *F. inermis* are mostly cultivated in home gardens; the excess crop is wasted and their economic potential as a health giving food crop has not been evaluated. *F. inermis* and *F. indica* are a source of proteins, lipids, vitamins, minerals, carbohydrates, terpenoids, saponins, alkaloids, anthraquinones and phenolic antioxidants such as chlorogenic acids (CGAs), flavonoids, anthocyanins, benzoyl glucosides and hydroxybenzyl alcohol glycosides (Amarasinghe, Jayasinghe, Hara, & Fujimoto, 2007; Dehmlow, Guntenhoner, & van Ree, 2000; Jayasinghe, Lakdusinghe, Hara, & Fujimoto, 2012; Madan, Pannakal, Ganapaty, Singh, & Kumar, 2009; Ndhlala et al., 2007; Sashidhara et al., 2013; Tchibozo, Savadogo, Karou, Toukourou, & de Souza, 2012). Amarasinghe et al. reported flacourside, 4-oxo-2-cyclopentenylmethyl 6-*O*-(*E*)-*p*-coumaroyl-β-D-glucopyranoside **27** and 6-*O*-(*E*)-*p*-coumaroyl glucopyranose from the fruits of *F. indica* (Amarasinghe et al., 2007). Recently, we reported (*rel*)-6*a*-benzoyloxy-1*a*,2*a*-dihydroxy-5-oxocyclohex-3-enecarboxylic acid 2-(6-*O*-benzoyl-*b*-D-glucopyranosyloxy)-5-hydroxybenzyl ester, methyl 3-*O*-caffeoylquinate **17**, methyl 5-*O*-caffeoylquinate **19**, methyl 4-*O*-caffeoylquinate **18**, butyl 3-*O*-caffeoylquinate, butyl 5-*O*-caffeoylquinate, together with quinic

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acid and malic acid from the fruits of *F. inermis* (Jayasinghe et al., 2012). *F. indica* fruit is used in folk medicine to treat jaundice and enlarged spleens. Due to the presence of saponins, β-sitosterols in *F. indica* fruit are not recommended to be consumed during the pregnancy and sitosterolemia. *F. indica* fruit contains a high level of serotonin and it is also not recommended to be consumed 72 h before the carcinoid tumor detection which may cause a false detection of cancer tumor. Preliminary investigations indicated the presence of the phenolic compounds, chlorogenic acid glycosides and cinnamoyl-hexoses present in *F. indica* and *F. inermis* fruits. CGA glycosides are an interesting challenge from an analytical perspective since their pseudomolecular ions are isobaric (same *m/z* value) to caffeoylquinic acids. Another interesting analytical challenge is to assign the regio- and stereochemistry of caffeoyl-, *p*-coumaroyl- and feruloyl glucoses by various LC-MS methods. In our previous studies we have developed LC-MS$^n$ methods for the positive identification and characterization of phenolic compounds from food materials and beverages (Jaiswal, Kiprotich, & Kuhnert, 2011; Jaiswal & Kuhnert, 2010; Jaiswal & Kuhnert, 2011b; Jaiswal, Patras, Eravuchira, & Kuhnert, 2010; Jaiswal, Sovdat, Vivan, & Kuhnert, 2010). In this contribution we are developing new LC-MS$^n$ methods for the identification and characterization of *F. indica* and *F. inermis* fruits phenolic compounds especially, chlorogenic acid glucosides, caffeoyl glucoses and *p*-coumaroyl glucoses.

## Materials and methods

All the chemicals and authentic standards were purchased from Sigma-Aldrich, PhytoLab and Carl-Roth (Bremen, Germany). Fruits of *F. indica* and *F. inermis* were collected from the Central Province of Sri Lanka in summer 2011. Quercetin **9**, rutin **10**, kaempferol-3-*O*-rutinoside **15**, 3-*O*-caffeoylquinic acid (3-CQA) **1**, 4-*O*-caffeoylquinic acid (4-CQA) **3**, 5-*O*-caffeoylquinic acid (5-CQA) **4**, 3,4-di-*O*-caffeoylquinic acid (3,4-diCQA) **6**, 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA) **7**, 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA) **8**, esculin **24**, quercetin-3-*O*-galactoside **11** and quercetin-3-*O*-glucoside **12** were used as authentic standards.

### *Methanolic extract of Flacourtia fruits*

Fruits of *F. indica* and *F. inermis* (5 g) were crushed and extracted with aqueous methanol (70%, 100 mL) using ultra-sonication for 30 min. This extract was filtered through a Whatman no. 1 filter paper. The methanol and the water were removed in vacuo and the residue was stored at −20 °C until required, thawed at room temperature, dissolved in methanol (5 mg/mL), filtered through a membrane filter and then used for LC-MS.

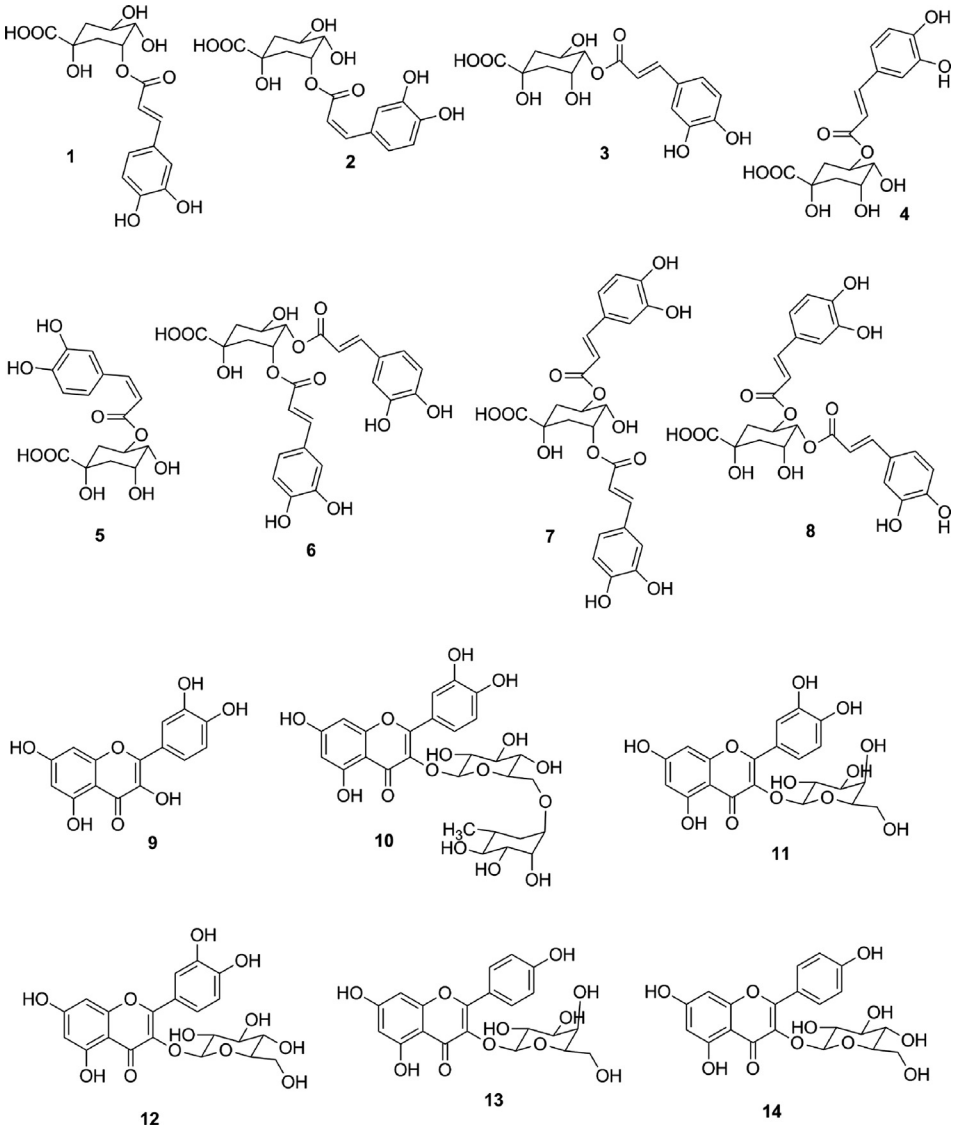


**Fig. 1.** Representative structures of *F. indica* and *F. inermis* fruits phenolics.

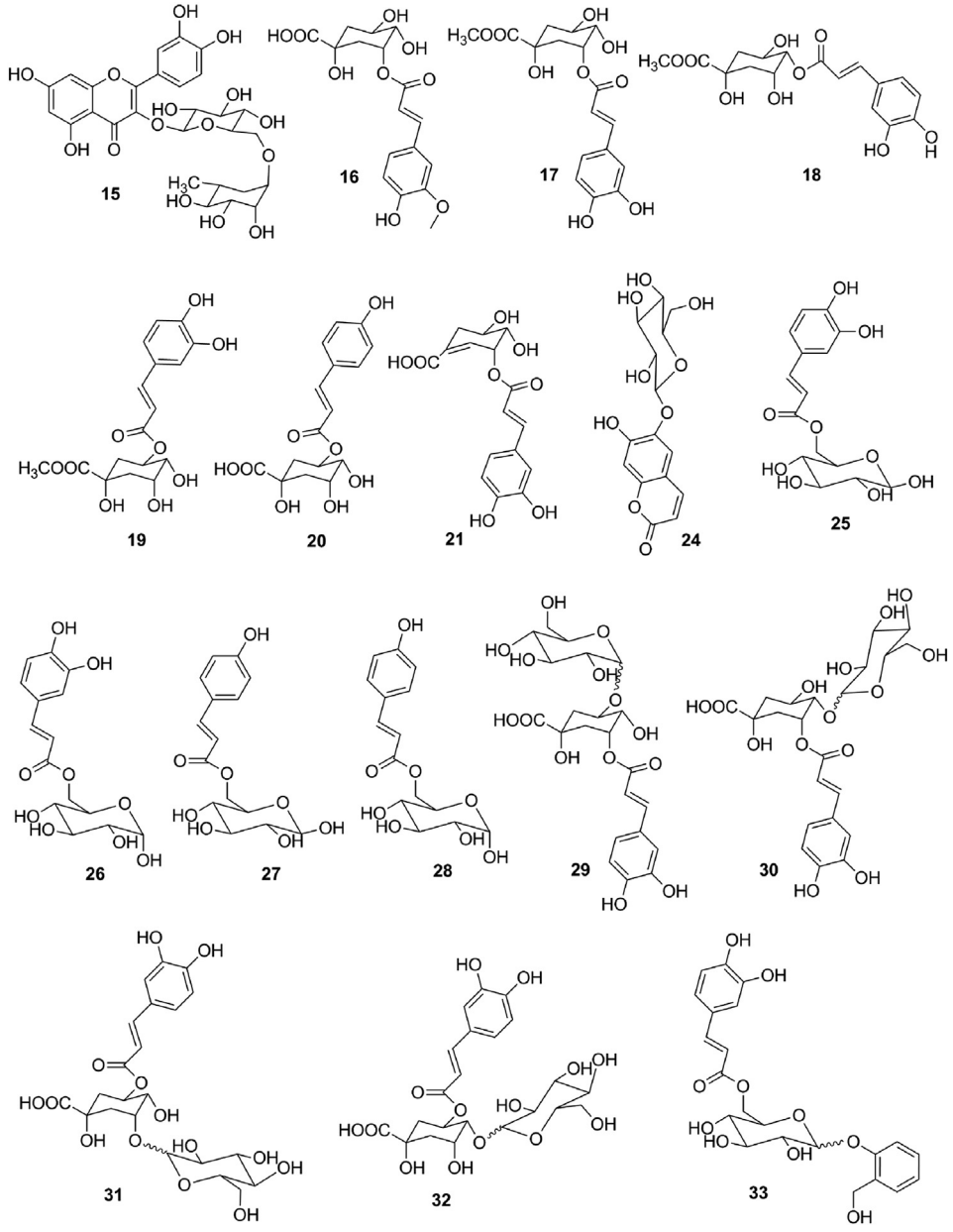


**Fig. 1** (*continued*).

*LC-MS$^n$*

The LC equipment (Agilent, Karlsruhe, Germany) comprised a binary pump, an auto sampler with a 100 μL loop and a DAD detector with a light-pipe flow cell (recording at 254, 280 and 320 nm and scanning from 200 to 600 nm). This was interfaced with an ion-trap mass spectrometer fitted with an ESI source (Bruker Daltonics, Bremen, Germany) operating in full scan, Auto MS$^n$ mode to obtain fragment ion *m/z*. As necessary, MS$^2$, MS$^3$ and MS$^4$ fragment-targeted experiments were performed. Tandem mass spectra were acquired in the auto-MS$^n$ mode (smart fragmentation) using a ramping of the collision energy. Maximum fragmentation amplitude was set to 1 V, starting at 30% and ending at 200%. The MS operating conditions (negative ion mode) had been optimized using 3-CQA **1**, 5-CQA **4** and rutin **10** with a capillary temperature of 365 °C, a dry gas flow rate of 10 L/min and a nebulizer pressure of 10 psi. High resolution LC-MS was carried out using the same HPLC equipped with a micrOTOF focus mass spectrometer (Bruker Daltonics, Bremen, Germany) fitted with an ESI source and internal calibration was achieved with 10 mL of a 0.1 M sodium formate solution injected through a six port valve prior to each chromatographic run. Calibration was carried out using the enhanced quadratic mode and the mass error was below 5 ppm (Table 1).

*HPLC coupled to MS*

Separation was achieved on a 250 x 3 mm inner-diameter column containing 5 μm C18 amide, with a 5 mm × 3 mm inner-diameter guard column (Varian, Darmstadt, Germany). Solvent A was water/formic acid (1000:0.005 v/v) and solvent B was methanol. Solvents were delivered at a total flow rate of 500 μL/min. In the HPLC method the gradient profile was from 10% B to 70% B linearly in 60 min followed by 10 min isocratic and a return to 10% B at 90 min and finally 10 min isocratic to re-equilibrate (Jaiswal & Kuhnert, 2014).

*NMR*

$^1$H and $^{13}$C NMR spectra were recorded on a JEOL-ECX 400 spectrometer operating at 400 MHz for $^1$H NMR and 100 MHz for $^{13}$C NMR at

**Table 1**
High resolution MS data of *F. indica* and *F. inermis*.

| No. | Compound | Molecular formula | Theoretical *m/z* (M − H) | Experimental *m/z* (M − H) for *F. indica* | Error (ppm) | Experimental *m/z* (M − H) for *F. inermis* | Error (ppm) |
|---|---|---|---|---|---|---|---|
| **1** | 3-*O*-caffeoylquinic acid | $C_{16}H_{18}O_9$ | 353.0878 | 353.0893 | −4.1 | 353.0895 | −4.9 |
| **2** | *Cis* 3-*O*-caffeoylquinic acid | $C_{16}H_{18}O_9$ | 353.0878 | 353.0894 | −4.7 | 353.0889 | −3.2 |
| **3** | 4-*O*-caffeoylquinic acid | $C_{16}H_{18}O_9$ | 353.0878 | 353.0892 | −4.0 | 353.0894 | −4.4 |
| **4** | 5-*O*-caffeoylquinic acid | $C_{16}H_{18}O_9$ | 353.0878 | 353.0888 | −2.8 | 353.0887 | −2.4 |
| **5** | *Cis* 5-*O*-caffeoylquinic acid | $C_{16}H_{18}O_9$ | 353.0878 | 353.0890 | −3.4 | 353.0888 | −2.8 |
| **6** | 3,4-Di-*O*-caffeoylquinic acid | $C_{25}H_{24}O_{12}$ | 515.1195 | 515.1216 | −4.1 | 515.1203 | −1.6 |
| **7** | 3,5-Di-*O*-caffeoylquinic acid | $C_{25}H_{24}O_{12}$ | 515.1195 | 515.1221 | −5.0 | 515.1219 | −4.6 |
| **8** | 4,5-Di-*O*-caffeoylquinic acid | $C_{25}H_{24}O_{12}$ | 515.1195 | 515.1196 | −0.1 | 515.1192 | 0.5 |
| **9** | Quercetin | $C_{15}H_{10}O_7$ | 301.0354 | – | – | 301.0357 | −0.9 |
| **10** | Rutin | $C_{27}H_{30}O_{16}$ | 609.1461 | 609.1486 | −4.1 | 609.1461 | −4.8 |
| **11** | Quercetin-3-*O*-galactoside | $C_{21}H_{20}O_{12}$ | 463.0882 | – | – | 463.0904 | −4.8 |
| **12** | Quercetin-3-*O*-glucoside | $C_{21}H_{20}O_{12}$ | 463.0882 | 463.0904 | −4.8 | 463.0898 | −3.5 |
| **13** | Kaempferol-3-*O*-galactoside | $C_{21}H_{20}O_{11}$ | 447.0933 | – | – | 447.0947 | −3.1 |
| **14** | Kaempferol-3-*O*-glucoside | $C_{21}H_{20}O_{11}$ | 447.0933 | – | – | 447.0933 | −4.9 |
| **15** | Kaempferol-3-*O*-rutinoside | $C_{27}H_{30}O_{15}$ | 593.1512 | – | – | 593.1531 | −3.2 |
| **16** | 3-*O*-feruloylquinic acid | $C_{17}H_{20}O_9$ | 367.1035 | 367.1048 | −3.6 | 367.1047 | −3.3 |
| **17** | Methyl 3-*O*-caffeoylquinic acid | $C_{17}H_{20}O_9$ | 367.1035 | 367.1049 | −3.8 | 367.1043 | −2.4 |
| **18** | Methyl 4-*O*-caffeoylquinic acid | $C_{17}H_{20}O_9$ | 367.1035 | – | – | 367.1049 | −4.0 |
| **19** | Methyl 5-*O*-caffeoylquinic acid | $C_{17}H_{20}O_9$ | 367.1035 | – | – | 367.1049 | −4.0 |
| **20** | 3-*O*-*p*-Coumaroylquinic acid | $C_{16}H_{18}O_8$ | 337.0929 | 337.0937 | −2.5 | 337.0927 | 0.5 |
| **21** | 3-*O*-Caffeoylshikimic acid | $C_{16}H_{16}O_8$ | 335.0772 | 335.0783 | −3.2 | 335.0785 | −3.8 |
| **22** | Caffeoylshikimic acid | $C_{16}H_{16}O_8$ | 335.0772 | 335.0778 | −1.5 | 335.0784 | −3.5 |
| **23** | Caffeoylshikimic acid | $C_{16}H_{16}O_8$ | 335.0772 | 335.0783 | −3.2 | 335.0787 | −4.5 |
| **24** | Esculin | $C_{15}H_{16}O_9$ | 339.0722 | – | – | 339.0722 | 0.0 |
| **25** | 6-*O*-caffeoyl-β-glucose | $C_{15}H_{18}O_9$ | 341.0878 | 341.0883 | −1.6 | – | – |
| **26** | 6-*O*-caffeoyl-α-glucose | $C_{15}H_{18}O_9$ | 341.0878 | 341.0893 | −4.3 | – | – |
| **27** | 6-*O*-*p*-coumaroyl-β-glucose | $C_{15}H_{18}O_8$ | 325.0929 | 325.0937 | −2.6 | – | – |
| **28** | 6-*O*-*p*-coumaroyl-α-glucose | $C_{15}H_{18}O_8$ | 325.0929 | 325.0936 | −2.1 | – | – |
| **29** | 3-*O*-caffeoyl-5-*O*-glucosylquinic acid | $C_{22}H_{28}O_{14}$ | 515.1406 | 515.1429 | −4.3 | 515.1415 | −1.8 |
| **30** | 3-*O*-caffeoyl-4-*O*-glucosylquinic acid | $C_{22}H_{28}O_{14}$ | 515.1406 | 515.1432 | −5.0 | 515.1423 | −3.3 |
| **31** | 3-*O*-glucosyl-5-*O*-caffeoylquinic acid | $C_{22}H_{28}O_{14}$ | 515.1406 | 515.1424 | −3.5 | 515.1405 | 0.2 |
| **32** | 4-*O*-glucosyl-5-*O*-caffeoylquinic acid | $C_{22}H_{28}O_{14}$ | 515.1406 | 515.1421 | −2.8 | 515.1402 | 0.8 |
| **33** | 2-*O*-(6′-*O*-glucosyl caffeoyl)-2-hydroxybenzyl alcohol | $C_{22}H_{24}O_{10}$ | 447.1297 | 447.1317 | −4.6 | – | – |
| **34** | Gentisic acid glycoside | $C_{13}H_{16}O_9$ | 315.0722 | 315.0730 | −2.8 | 315.0725 | −1.0 |
| **35** | Salicylic acid glycoside | $C_{13}H_{16}O_8$ | 299.0772 | 299.0777 | −1.5 | 299.0757 | 5.0 |

room temperature in acetone-$d_6$ or methanol-$d_4$ using a 5 mm probe. The chemical shifts ($\delta$) are reported in ppm and were referenced to the residual solvent peak. The coupling constants ($J$) are quoted in Hz.

*Synthesis of 6-O-caffeoyl glucoses (**25** and **26**)*

We have synthesized caffeoyl glucoses **25** and **26** by a method described by Jaiswal and Kuhnert (2014). Pyridine (5 mL, 62 mmol) and 3,4-di-*O*-allylcaffeoyl chloride (1.58 g, 5.70 mmol) were added to a solution of 1,2-*O*-isopropylidene-α-D-glucofuranose (1.00 g, 4.54 mmol) and DMAP (200 mg, 1.63 mmol) in DCM (50 mL) (Jaiswal, Dickman, & Kuhnert, 2012) at room temperature. The reaction mixture was stirred for 24 h at room temperature and acidified (pH ≈ 3) with 2 M HCl. The layers were separated and the aqueous phase was extracted with DCM (3 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered and the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (ethyl acetate-petroleum ether, 30-50%) to give 6-*O*-(3,4-di-*O*-allyl)caffeoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (80%) (Jaiswal & Kuhnert, 2014).

$\delta_H$ (acetone-$d_6$): 7.62 (1H, d, $J$ 15.6, $C_{Ar}-CH$), 7.32 (1H, dd, $J$ 1.8, $C_{Ar}H$), 7.17 (1H, dd, $J$ 8.7, 2.3 $C_{Ar}H$), 6.99 (1H, d, $J$ 8.2, $C_{Ar}H$), 6.41 (1H, d, $J$ 16.0, $C_{Ar}-CH{=}CH$), 6.08 (2H, m, $CH_2{=}CH$), 5.85 (1H, d, $J$ 3.6, $H$-3), 5.43 (2H, m, $CHH{=}CH$), 5.23 (2H, m, $CHH{=}CH$), 4.65 (4H, m, $C_{Ar}-OCH_2$), 4.49 (1H, d, $J$ 3.7, $H$-2), 4.31 (2H, m, $H$-6a, $H$-6b), 4.12 (2H, m, $H$-4, $H$-5), 1.39 (3H, s, $OCH_3$), 1.24 (3H, s, $OCH_3$); $\delta_C$ (acetone-$d_6$): 166.6 ($-COOC$), 150.9 ($C_{Ar}-OCH_2$), 148.9 ($C_{Ar}-OCH_2$), 144.5 ($C_{Ar}-CH$), 133.9 ($CH_2{=}CH$), 133.5 ($CH_2{=}CH$), 127.7 ($C_{Ar}-CH$), 122.9 ($C_{Ar}$), 116.9 ($CH_2{=}CH$), 116.5 ($CH-COO$), 115.7 ($C_{Ar}$), 113.7 ($C_{Ar}$), 112.6 ($H_3C-O-C-O-CH_3$), 111.2 ($H_3C-O-C-O-CH_3$), 105.0 ($C$-3), 85.3 ($C$-6), 80.4 ($C$-1), 74.4 ($C$-2), 69.4 ($C$-4), 69.2 ($C_{Ar}-OCH_2$), 67.1 ($C_{Ar}-OCH_2$), 66.8 ($C$-5), 26.4 ($OCH_3$), 25.6 ($OCH_3$) (Jaiswal & Kuhnert, 2014).

10% Pd/C (350 mg) was added to a solution of 6-*O*-(3,4-di-*O*-allyl) caffeoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (1.00 g, 2.23 mmol) and *p*-TsOH (50 mg) in methanol–water (9:1, 50 mL) at room temperature. The reaction mixture was heated at 70 °C for 48 h, cooled to room temperature, and filtered, and methanol was removed in vacuo. The aqueous reaction mixture was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over sodium sulfate, filtered and the solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel (ethyl acetate-petroleum ether, 50–95%) to give 6-*O*-caffeoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (62%). The resulting ester 6-*O*-caffeoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (400 mg, 1.10 mmol) was dissolved in a mixture of 20 mL of TFA and water (8:2) at room temperature and stirred for 1 h. The solvents were removed in vacuo to obtain the resulting esters 6-*O*-caffeoyl glucoses (**25** and **26**) in quantitative yield (Jaiswal & Kuhnert, 2014).

$\delta_H$ (methanol-$d_3$): 7.54 (1H, d, $J$ 16.0, $C_{Ar}-CH$), 7.02 (1H, d, $J$ 2.2, $C_{Ar}-H$), 6.92 (1H, dd, $J$ 8.2, 1.4, $C_{Ar}-H$), 6.75 (1H, d, $J$ 8.2, $C_{Ar}-H$), 6.25 (1H, $J$ 16.5, $C_{Ar}-CH{=}CH$), 5.10 (1H, d, $J$ 3.7, gluc $H$-1), 4.45 (1H, m, gluc $H$-6a), 4.30 (1H, m, gluc $H$-6b), 4.05 (1H, m, gluc $H$-4), 3.28–3.37 (2H, m, gluc $H$-2, gluc $H$-5); $\delta_C$ (methanol-$d_3$): 167.9 ($COOC$), 148.3 ($C_{Ar}$), 145.8 ($C_{Ar}-CH$), 145.5 ($C_{Ar}$), 126.4 ($C_{Ar}$), 123.6 ($C_{Ar}$), 121.6 ($C_{Ar}$), 115.2 ($CH_2{=}CH$), 113.7 ($CH-COO$), 113.6 ($CH_2{=}CH$), 96.8 (β-gluc $C$-1), 92.6 (α-gluc $C$-1), 76.7 (β-gluc $C$-3), 74.9 (β-gluc $C$-2), 74.1 (β-gluc $C$-5), 73.5 (α-gluc $C$-3), 72.2 (α-gluc $C$-2), 71.6 (α-gluc $C$-5), 70.4 (α-gluc $C$-4), 69.6 (β-gluc $C$-4), 63.6 (β-gluc $C$-6), 63.5 (α-gluc $C$-6) (Jaiswal & Kuhnert, 2014).

## Results and discussion

### *Preliminary assessment of data*

All the data for CGAs and flavonoids presented in this paper use the recommended IUPAC (1976) numbering system and nomenclature suggested by Porter (1988). When necessary, previously published data has been amended to ensure consistency and avoid ambiguity. Not all the phenolics reported here were present in both samples (Table 1). In general, CGAs and their derivatives can be identified in an all tandem mass spectrum EIC (extracted ion chromatogram) by their unique fragments at *m/z* 173 and 191. Selected ion monitoring at *m/z* 335, 337, 353, 367 and 529 immediately located twenty chromatographic peaks eluting between 13 and 42 min, each with a UV spectrum typical of CGAs and cinnamates [$\lambda_{max}$ 320 nm]. During this study we did not observe any aliphatic acid-containing monoacyl, diacyl and triacyl or tetraacyl quinic acid isomer (Clifford, Wu, Kirkpatrick, Jaiswal, & Kuhnert, 2010; Jaiswal & Kuhnert, 2010; Jaiswal & Kuhnert, 2011b; Jaiswal & Kuhnert, 2011c; Jaiswal, Patras et al., 2010; Jaiswal, Sovdat et al., 2010). Presence of the glucosyl residue was confirmed by acid and base hydrolysis of the samples followed by LC–MS analysis. We have observed that flavonol 3-*O*-galactoside elutes first followed by flavonol 3-*O*-glucoside (where $\Delta t_r < 1.5$ min).

### *Characterization of mono caffeoylquinic acids (Mr 354) and dicaffeoylquinic acids (Mr 516)*

Five caffeoylquinic acids eluting from ~13 to 24 min were easily located and assigned using the hierarchical keys previously developed (Clifford, Johnston, Knight, & Kuhnert, 2003; Clifford, Knight, & Kuhnert, 2005) as the well-known 3-*O*-caffeoylquinic acid (**1**), *cis*-3-*O*-caffeoylquinic acid (**2**), 4-*O*-caffeoylquinic acid (**3**), 5-*O*-caffeoylquinic acid (**4**) and *cis* 5-*O*-caffeoylquinic acid (**5**) (Table 2). Detailed mass spectra were published previously and are not repeated here (Clifford et al., 2003, 2005). In our previous studies we reported the presence of *cis* isomers of the CGAs in plants leaves, which resulted from *cis-trans* isomerization in the presence of UV light (Clifford, Kirkpatrick, Kuhnert, Roozendaal, & Salgado, 2008; Jaiswal, Deshpande, & Kuhnert, 2011; Jaiswal, Kiprotich, & Kuhnert, 2011).

Three dicaffeoylquinic acids were detected at *m/z* 515 in EIC and were assigned using the hierarchical keys previously developed as 3,4-di-*O*-caffeoylquinic acid (**6**), 3,5-di-*O*-caffeoylquinic acid (**7**) and 4,5-di-*O*-caffeoylquinic acid (**8**) (Table 2).

### *Identification of flavonoids and flavonoid-glycosides*

One peak was detected at *m/z* 301 in the extracted ion chromatogram and was assigned as quercetin **9**. One peak was detected at *m/z* 609 and was assigned as rutin **10**. It produced the $MS^2$ base peak at *m/z* 300 ($[M - H^+ - 309\ Da]^-$) by the loss of the rutinoside moiety attached to quercetin at C-3 (Table 2). The retention time and fragmentation pattern were identical to an authentic standard of rutin **10**.

Two peaks were detected at *m/z* 463 in the extracted ion chromatogram and were tentatively assigned as quercetin-hexosides (**11** and **12**). Both isomers had similar $MS^n$ fragmentation patterns with similar ion intensities (Table 2). They produced the $MS^2$ base peak at *m/z* 300 ($[M - H^+ - 163\ Da]^-$) by a neutral loss of a 162 Da (Table 2) hexoside, which is characteristic of quercetin-3-*O*-hexoside. They produced the $MS^3$ base peak at *m/z* 151 and secondary peaks at *m/z* 179, 271 and 255 which are characteristics of a quercetin moiety (Table 2). It was not possible to differentiate between these isomers by tandem mass spectra in negative ion mode. Based on their retention times and comparing to the authentic standards of quercetin-3-*O*-galactoside **11** and quercetin-3-*O*-glucoside **12**, the first eluting isomer was assigned as quercetin-3-*O*-galactoside **11** and the second eluting isomer was assigned as quercetin-3-*O*-glucoside **12**.

Two peaks were detected at *m/z* 447 in the extracted ion chromatogram and were tentatively assigned as kaempferol-glucosides (**13** and **14**). Both isomers have similar $MS^n$ fragmentation patterns with similar ion intensities (Table 2). They produced the $MS^2$ base peak at *m/z* 285 ($[M - H^+ - 162\ Da]^-$) by a neutral loss of a 162 Da (Table 2) glucoside, which is characteristic of kaempferol-*O*-glucoside. They produced the $MS^3$ base peak at *m/z* 255 and secondary peaks at *m/z* 227 and 241 which are characteristics of a kaempferol moiety. Based on the fragmentation pattern, authentic standard and order of elution, isomers **13** and **14** were assigned as kaempferol-3-*O*-galactoside and kaempferol-3-*O*-glucoside, respectively.

One peak was detected at *m/z* 593 in the extracted ion chromatogram and was tentatively assigned as kaempferol-rutinoside (**15**). It produced the $MS^2$ base peak at *m/z* 285 ($[M - H^+ - 146\ Da - 162\ Da]^-$) by a neutral loss of a 146 Da rhamnoside and 162 Da glucoside moieties (Table 2). It produced the $MS^3$ base peak at *m/z* 255 and secondary peaks at *m/z* 227 and 241 which are characteristics of a kaempferol moiety. Based on the MS fragmentation and authentic standard of kaempferol-3-*O*-rutinoside **15** this compound was assigned as kaempferol-3-*O*-rutinoside.

### *Characterization of feruloylquinic acid (Mr 368) and methyl caffeoylquinates (Mr 368)*

The feruloylquinic acid isomer **16** eluting at ~21 min was assigned as 3-feruloylquinic acid (Table 1). Three peaks located (~20–29 min) in the extracted ion chromatogram produced each the pseudomolecular ion at *m/z* 367 (**17**–**19**) and were tentatively assigned as methyl caffeoylquinates. The first (**17**) and the second (**18**) eluting isomer produced the $MS^2$ base peak at *m/z* 161 ([caffeic acid-$H_2O$-$H^+$]$^-$) and an $MS^2$ secondary peak at *m/z* 135 ([caffeic acid-$CO_2$-$H^+$]$^-$) (Table 1). The third eluting isomer (**19**) produced the $MS^2$ base peak at *m/z* 179 and $MS^2$ secondary peaks at *m/z* 135 ([caffeic acid-$CO_2$-$H^+$]$^-$), 161 ([caffeic acid-$H_2O$-$H^+$]$^-$) and 191 ([quinic acid-$H^+$]$^-$) (Table 2) (Jaiswal & Kuhnert, 2011a). It produced the $MS^3$ base peak at *m/z* 135 ([caffeic acid-$CO_2$-$H^+$]$^-$) by the loss of a $CO_2$ molecule. The retention times and fragmentation patterns of these isomers (**17**–**19**) were compared to the previously studied methyl caffeoylquinates and were assigned as methyl 3-*O*-caffeoylquinate, methyl 4-*O*-caffeoylquinate and methyl 5-*O*-caffeoylquinate, respectively (Jaiswal & Kuhnert, 2011a). Recently, we isolated compounds **17**–**19** from *F. inermis* fruit juice (Jayasinghe et al., 2012).

### *Characterization of p-coumaroylquinic acid (Mr 338)*

One peak detected in the extracted ion chromatogram produced a pseudomolecular ion at *m/z* 337 and the $MS^2$ base peak at *m/z* 191 ([quinic acid-$H^+$]$^-$) which are characteristics of 5-*O*-*p*-coumaroylquinic acid **20** (Table 2) (Jaiswal, Patras et al., 2010; Jaiswal, Sovdat et al., 2010).

### *Characterization of caffeoyl-shikimates (Mr 336)*

Three peaks detected in the extracted ion chromatogram produced a pseudomolecular ion at *m/z* 335. The first eluting isomer **21** produced the $MS^2$ base peak at *m/z* 179 ([caffeic acid-$H^+$]$^-$) and a secondary peak at *m/z* 135 (Table 2). Based on the fragmentation pattern isomer **13** was assigned as 3-*O*-caffeoylshikimic acid. Isomers **22** and **23** were tentatively assigned as caffeoylshikimic acids despite the lack of authentic standards; they showed however different $MS^2$ and $MS^3$ spectra (Table 2) if compared to the $MS^2$ and $MS^3$ spectra of caffeoylshikimic acids previously reported in maté tea (Jaiswal, Patras et al., 2010; Jaiswal, Sovdat et al., 2010).

**Table 2**
Negative ion $MS^4$ fragmentation data for the phenolics of *F. indica* and *F. inermis*.

| No. | Phenolics | Retention time (min) | Parent ion *m/z* (M − H) | Characteristic *m/z* of ions in negative ion mode |
|---|---|---|---|---|
| **1** | 3-*O*-caffeoylquinic acid | 13.1 | 353 | $MS^2$ → 191 (100), 179 (46), 135 (11); $MS^3$ → 127 (100), 173 (58), 109 (29), 85 (67) |
| **2** | *Cis* 3-*O*-caffeoylquinic acid | 14.1 | 353 | $MS^2$ → 191 (100), 179 (45), 135 (9); $MS^3$ → 127 (100), 173 (85), 111 (67), 85 (60) |
| **3** | 4-*O*-caffeoylquinic acid | 23.5 | 353 | $MS^2$ → 173 (100), 191 (36), 179 (59); $MS^3$ → 93 (100), 173 (20), 111 (31) |
| **4** | 5-*O*-caffeoylquinic acid | 18.5 | 353 | $MS^2$ → 191 (100); $MS^3$ → 127 (100), 173 (94), 111 (29), 93 (58), 85 (82) |
| **5** | *Cis* 5-*O*-caffeoylquinic acid | 24.0 | 353 | $MS^2$ → 191 (100); $MS^3$ → 127 (100), 173 (92), 111 (27), 93 (60), 85 (78) |
| **6** | 3,4-Di-*O*-caffeoylquinic acid | 40.0 | 515 | $MS^2$ → 353 (100), 335 (17), 203 (7), 191 (12), 179 (12), 173 (33); $MS^3$ → 173 (100), 191 (38), 179 (57) |
| **7** | 3,5-Di-*O*-caffeoylquinic acid | 38.4 | 515 | $MS^2$ → 353 (100), 335 (5), 299 (9), 255 (6), 203 (13), 179 (10), 173 (16); $MS^3$ → 173 (100), 191 (45), 179 (40) |
| **8** | 4,5-Di-*O*-caffeoylquinic acid | 42.0 | 515 | $MS^2$ → 353 (100); $MS^3$ → 191 (100), 179 (48), 135 (16) |
| **9** | Quercetin | 45.8 | 301 | $MS^2$ → 179 (100), 271 (50), 255 (30), 151 (70) |
| **10** | Rutin | 36.3 | 609 | $MS^2$ → 301 (100); $MS^3$ → 179 (100), 271 (53), 255 (39), 151 (97) |
| **11** | Quercetin-3-*O*-galactoside | 35.6 | 463 | $MS^2$ → 301 (100); $MS^3$ → 179 (100), 271 (45), 255 (24), 151 (84) |
| **12** | Quercetin-3-*O*-glucoside | 36.6 | 463 | $MS^2$ → 301 (100); $MS^3$ → 179 (100), 271 (50), 255 (33), 151 (80) |
| **13** | Kaempferol-3-*O*-galactoside | 38.7 | 447 | $MS^2$ → 285 (100); $MS^3$ → 255 (100), 227 (13) |
| **14** | Kaempferol-3-*O*-glucoside | 40.2 | 447 | $MS^2$ → 285 (100); $MS^3$ → 255 (100), 227 (15) |
| **15** | Kaempferol-3-*O*-rutinoside | 40.2 | 593 | $MS^2$ → 285 (100); $MS^3$ → 255 (100), 267 (46), 241 (36), 229 (37), 213 (30) |
| **16** | 3-*O*-feruloylquinic acid | 21.6 | 367 | $MS^2$ → 193 (100), 134 (12); $MS^3$ → 134 (100), 149 (18) |
| **17** | Methyl 3-*O*-caffeoylquinic acid | 20.1 | 367 | $MS^2$ → 161 (100), 335 (7), 193 (15), 135 (10); $MS^3$ → 133 (100) |
| **18** | Methyl 4-*O*-caffeoylquinic acid | 27.8 | 367 | $MS^2$ → 161 (100), 335 (32), 193 (9), 135 (5); $MS^3$ → 133 (100) |
| **19** | Methyl 5-*O*-caffeoylquinic acid | 29.0 | 367 | $MS^2$ → 179 (100), 191 (23), 161 (13), 135 (45); $MS^3$ → 135 (100) |
| **20** | 3-*O*-*p*-coumaroylquinic acid | 25.2 | 337 | $MS^2$ → 191 (100), 163 (5); $MS^3$ → 127 (100), 173 (94), 111 (29), 93 (58), 85 (82) |
| **21** | 3-*O*-caffeoylshikimic acid | 25.7 | 335 | $MS^2$ → 179 (100), 161 (9), 135 (44); $MS^3$ → 135 (100) |
| **22** | Caffeoylshikimic acid | 26.2 | 335 | $MS^2$ → 161 (100), 179 (10), 135 (7); $MS^3$ → 133 (100) |
| **23** | Caffeoylshikimic acid | 30.0 | 335 | $MS^2$ → 161 (100), 255 (8), 179 (13), 135 (16); $MS^3$ → 133 (100) |
| **24** | Esculin | 24.0 | 339 | $MS^2$ → 177 (100); $MS^3$ → 133 (100) |
| **25** | 6-*O*-caffeoyl-β-glucose | 14.2 | 341 | $MS^2$ → 281 (100), 323 (5), 251 (79), 221 (16), 179 (57), 135 (4); $MS^3$ → 179 (100), 221 (35), 135 (15) |
| **26** | 6-*O*-caffeoyl-α-glucose | 16.9 | 341 | $MS^2$ → 281 (100), 323 (5), 251 (79), 221 (16), 179 (57), 135 (4); $MS^3$ → 179 (100), 221 (35), 135 (15) |
| **27** | 6-*O*-*p*-coumaroyl-β-glucose | 19.2 | 325 | $MS^2$ → 265 (100), 235 (95), 205 (12), 163 (19); $MS^3$ → 205 (100), 247 (7), 187 (11), 163 (45), 145 (17), 119 (12) |
| **28** | 6-*O*-*p*-coumaroyl-α-glucose | 22.1 | 325 | $MS^2$ → 265 (100), 235 (95), 205 (12), 163 (19); $MS^3$ → 205 (100), 247 (7), 187 (11), 163 (45), 145 (17), 119 (12) |
| **29** | 3-*O*-caffeoyl-5-*O*-glucosylquinic acid | 9.6 | 515 | $MS^2$ → 335 (100), 353 (66), 395 (5), 455 (42), 191 (6); $MS^3$ → 161 (100), 179 (12), 135 (77) |
| **30** | 3-*O*-caffeoyl-4-*O*-glucosylquinic acid | 11.1 | 515 | $MS^2$ → 335 (100), 425 (14), 353 (93), 395 (9), 455 (24), 191 (10); $MS^3$ → 161 (100), 179 (10), 135 (82) |
| **31** | 3-*O*-glucosyl-5-*O*-caffeoylquinic acid | 14.8 | 515 | $MS^2$ → 353 (100), 455 (88), 425 (40), 395 (49), 191 (26); $MS^3$ → 191 (100) |
| **32** | 4-*O*-glucosyl-5-*O*-caffeoylquinic acid | 16.0 | 515 | $MS^2$ → 353 (100), 455 (24), 425 (67), 395 (41), 191 (28), 407 (10); $MS^3$ → 191 (100) |
| **33** | 2-*O*-(6′-*O*-glucosyl caffeoyl)-2-hydroxybenzyl alcohol | 35.5 | 447 | $MS^2$ → 323 (100), 377 (8), 263 (5), 221 (21), 179 (17), 161 (24), 135 (5); $MS^3$ → 179 (100), 263 (36), 221 (85), 161 (39), 135 (33) |
| **34** | Gentisic acid glycoside | 12.4 | 315 | $MS^2$ → 153 (100), 245 (40), 109 (10); $MS^3$ → 109 (100), 69 (52) |
| **35** | Salicylic acid glycoside | 8.8 | 299 | $MS^2$ → 137 (100), 239 (72), 209 (17), 179 (69); $MS^3$ → 93 (100) |

*Characterization of esculin (Mr 340)*

One peak was detected at *m/z* 339 in the extracted ion chromatograms and was assigned as esculin (**24**) after the comparison to the standard. It produced the $MS^2$ base peak at *m/z* 177 ([esculetin-$H^+$]$^-$) by the loss of a glucoside moiety and a secondary peak at *m/z* 133 ([esculetin-$CO_2$-$H^+$]$^-$) by the loss of a glucoside moiety and a $CO_2$ molecule (Table 2).

*Characterization of caffeoyl glucoses (Mr 342)*

Two peaks were detected at *m/z* 341 in the extracted ion chromatogram ($\lambda_{max}$ 320 nm) and were tentatively assigned as caffeoyl glucoses (**25** and **26**). They produced the $MS^2$ base peak at *m/z* 281 ([M − $H^+$−60 Da]$^-$) by the loss of $C_2H_4O_2$ and secondary peaks at *m/z* 251 ([M − $H^+$−90 Da]$^-$) by the loss of $C_3H_6O_3$, at *m/z* 221 ([M − $H^+$−120 Da]$^-$) by the loss of $C_4H_8O_4$ and at *m/z* 179 ([caffeic acid-$H^+$]$^-$) by the loss of a glucosyl residue ($C_6H_{10}O_5$) (Fig. 2 and Table 2) (Jaiswal & Kuhnert, 2014). Isomers **25** and **26** produced the ions ([M − $H^+$−60 Da]$^-$), ([M − $H^+$−90 Da]$^-$) and ([M − $H^+$−120 Da]$^-$) which are characteristics of glycosides (Jaiswal & Kuhnert, 2014).

Further evidence for the assignment of the caffeoyl glucoses came from an independent synthesis of 6-*O*-caffeoyl-β-glucose (**25**) and 6-*O*-caffeoyl-α-glucose (**26**). A β stereochemistry was assigned based on the characteristic of $^3J_{HCCH}$ coupling constant between the anomeric hydrogen at the hydrogen at C-2 of glucose, with 6-*O*-caffeoyl-α-glucose (**26**) showing values between 3 and 4 Hz and 6-*O*-caffeoyl-β-glucose (**25**) values between 7 and 8 Hz. On standing in methanol-$d_3$ the mixture of 6-*O*-caffeoyl-glucoses equilibrated, to yield after one day exclusively the β-anomers (Jaiswal & Kuhnert, 2014). The retention times and fragmentation pattern of synthetic 6-*O*-caffeoyl-glucoses were identical to isomers **25** and **26** reported in *F. indica*. Based on these arguments the first eluting isomer was assigned as 6-*O*-caffeoyl-β-glucose (**25**) and the later eluting isomer as 6-*O*-caffeoyl-α-glucose (**26**). Recently we have reported isomers **25** and **26** in the fruit of bottle gourd (Jaiswal & Kuhnert, 2014).

*Characterization of p-coumaroyl glucoses (Mr 326)*

Two peaks were detected at *m/z* 325 in the extracted ion chromatogram ($\lambda_{max}$ 320 nm) and were tentatively assigned as *p*-coumaroyl glucoses (**27** and **28**) (Jaiswal & Kuhnert, 2014). They produced the $MS^2$ base peak at *m/z* 265 ([M − $H^+$−60 Da]$^-$) by the loss of $C_2H_4O_2$, and secondary peaks at *m/z* 235 ([M − $H^+$−90 Da]$^-$) by the loss of $C_3H_6O_3$, at *m/z* 205 ([M − $H^+$−120 Da]$^-$) by the loss of $C_4H_8O_4$ and at *m/z* 163 ([*p*-coumaric acid-$H^+$]$^-$) by the loss of a glucosyl residue ($C_6H_{10}O_5$) (Fig. 2 and Table 2) (Jaiswal & Kuhnert, 2014). Isomers **27**

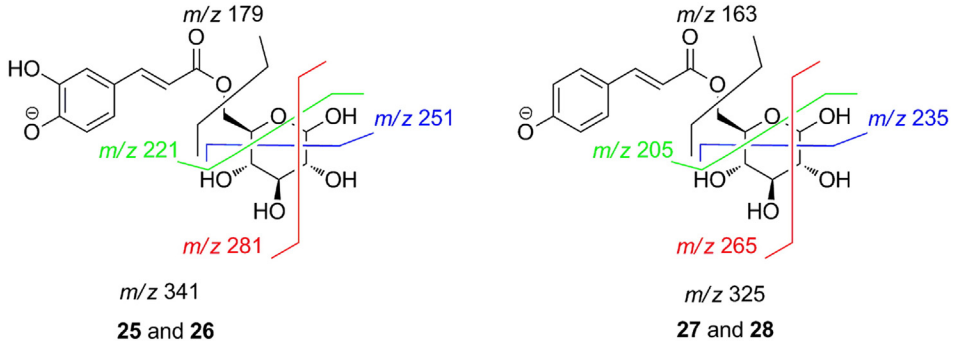


**Fig. 2.** Fragmentation pathways of 6-*O*-caffeoyl glucoses (**25** and **26**) and 6-*O*-*p*-coumaroyl glucoses (**27** and **28**) in negative ion mode.

and **28** produced the ions ([M − $H^+$−60 Da]$^-$), ([M − $H^+$−90 Da]$^-$) and ([M − $H^+$−120 Da]$^-$) which are characteristics of glycosides. Compounds **27** and **28** showed a fragmentation analogy to compounds **25** and **26**. Based on this argument isomer **27** was assigned as 6-*O*-*p*-coumaroyl-β-glucose and isomer **28** as 6-*O*-*p*-coumaroyl-α-glucose (Jaiswal & Kuhnert, 2014). Previously, Amarasinghe et al. isolated 6-*O*-*p*-coumaroyl-β-glucose from *F. indica* (Amarasinghe et al., 2007). Recently we have reported isomers **27** and **28** in the fruit of bottle gourd (Jaiswal & Kuhnert, 2014).

*Characterization of putative 3-O-caffeoylquinic acid glucosides and 5-O-caffeoylquinic acid glucosides (Mr 516)*

Four peaks were detected at *m/z* 515 in the extracted ion chromatogram and were tentatively assigned as caffeoylquinic acid glucosides (**29**–**32**). These compounds eluted early in the chromatogram, showed retention times similar to mono acyl CGAs and showed a typical UV absorbance of CGAs at 320 nm. High resolution MS spectra suggested the molecular formula $C_{22}H_{28}O_{14}$ (M − $H^+$) for these compounds which were caffeoylquinic acid glucosides (**29**–**32**). The first and second eluting isomers (**29** and **30**) produced the $MS^2$ base peak at *m/z* 335 ([caffeoylquinic acid-$H_2O$-$H^+$]$^-$) by the loss of a glucosyl residue (162 Da) and a $H_2O$ molecule; the secondary peaks were observed as following: the peak at *m/z* 353 ([caffeoylquinic acid-$H^+$]$^-$) by the loss of a glucosyl residue (162 Da), at *m/z* 191 ([quinic acid-$H^+$]$^-$) by the loss of a caffeoyl (162 Da) and a glucosyl residues (162 Da), at *m/z* 455 ([M-$C_2H_4O_2$-$H^+$]$^-$) by the loss of 60 Da, at *m/z* 395 ([M-$C_4H_8O_4$-$H^+$]$^-$) by the loss of 120 Da and at *m/z* 425 ([M-$C_3H_6O_3$-$H^+$]$^-$) by the loss of 90 Da (Fig. 3 and Table 2). They produced the $MS^3$ base peak at *m/z* 161 ([caffeic acid-$H_2O$-$H^+$]$^-$) by the loss of a quinic acid residue (174 Da) and secondary peaks at *m/z* 135 ([caffeic acid-$CO_2$-$H^+$]$^-$) by the loss of a quinic acid residue (174 Da) and $CO_2$ and at *m/z* 179 ([caffeic acid-$H^+$]$^-$) by the loss of a quinic acid part (156 Da) (Fig. 3 and Table 2). These compounds produced the characteristic fragment ions ([M − H+−60 Da]−), ([M − H+−90 Da]−) and ([M − H+−60 Da]−) of glycosides. (See Fig. 4.)

An MRM experiment of 515 → 353 produced the spectra identical to 3-*O*-caffeoylquinic acid (Fig. 3 and Table 2). The $MS^2$ peaks at *m/z* 341 [caffeoyl glucose-$H^+$]$^-$ and 323 [caffeoyl glucose-$H_2O$-$H^+$]$^-$ were absent which suggested that glucose was connected with the quinic acid moiety by an ether linkage and caffeic acid was connected with quinic acid at C-3 by an ester bond. It also ruled out the possibility of an ester linkage between glucoside –OH and quinic acid –COOH group. There are two possibilities for the glucosidic linkage, namely the C-4 and C-5 of quinic acid. In the absence of authentic standards it was not possible to assign the correct regiochemistry for these isomers. Based on the retention order of 3,5-diCQA and 3,4-diCQA on reverse phase stationary phase we assigned isomers **29** and **30** as 3-*O*-caffeoyl-5-*O*-glucosylquinic acid and 3-*O*-caffeoyl-4-*O*-glucosylquinic acid, respectively.

The third and the last eluting isomer (**31** and **32**) produced the $MS^2$ base peak at *m/z* 455 ([M-$C_2H_4O_2$-$H^+$]$^-$) and secondary peaks at *m/z* 425 ([M-$C_3H_6O_3$-$H^+$]$^-$), *m/z* 395 ([M-$C_4H_8O_4$-$H^+$]$^-$), *m/z* 353 ([caffeoylquinic acid-$H^+$]$^-$) and *m/z* 191 ([quinic acid-$H^+$]$^-$) (Fig. 3 and Table 2). They produced the $MS^3$ base peak at *m/z* 395 ([M-$C_4H_8O_4$-$H^+$]$^-$) and secondary peaks at *m/z* 437 ([M-$C_2H_4O_2$-$H_2O$-$H^+$]$^-$), *m/z* 353 ([caffeoylquinic acid-$H^+$]$^-$) and *m/z* 191 ([quinic acid-$H^+$]$^-$). An MRM experiment of 515 → 353 gave the base peak at *m/z* 191 ([quinic acid-$H^+$]$^-$) which is characteristic to 5-*O*-caffeoylquinic acid **4** (Table 2). From the above points it was clear that both isomers were 5-*O*-caffeoylquinic acid glucosides, with two possibilities for glucosyl connectivity, C-3 and C-4 of quinic acid. Similarly to **29** and **30**, these isomers were tentatively assigned as 3-*O*-glucosyl-5-*O*-caffeoylquinic acid **31** and 4-*O*-glucosyl-5-*O*-caffeoylquinic acid **32**.

Nikhat, Satynarayanaa, and Joshia (2008) reported a CGA glycoside, 4-*O*-pentosyl-5-*O*-caffeoylquinic acid from the roots of *Syzygium cuminii* (L.) skeel. This is the only CGA glycoside reported so far in which the pentosyl moiety is connected to a quinic acid moiety by an ether linkage (glycosidic bond). It is interesting to note that *Flacourtia* fruits contain a rare class of CGA glucosides which might be a characteristic of Flacourtiaceae family. This kind of characteristics has been observed for Rubiaceae family in which C-1 acylated CGAs are absent while in Asteraceae family C-1 acylated CGAs are present.

*Characterization of putative 4-O-(6′-O-glucosyl caffeoyl)-4-hydroxybenzyl alcohol (Mr 448)*

One peak was detected at *m/z* 447 which produced the $MS^2$ base peak at *m/z* 323 ([caffeoyl glucose-$H_2O$-$H^+$]$^-$) by the loss of the 2-hydroxybenzyl alcohol moiety (124 Da); it produced secondary peaks at *m/z* 179 ([caffeic acid-$H^+$]$^-$) by the loss of the 2-*O*-glucosyl-2-hydroxybenzyl alcohol residue (268 Da), at *m/z* 161 ([caffeic acid-$H_2O$-$H^+$]$^-$) by the loss of the 2-*O*-glucosyl 2-hydroxybenzyl alcohol moiety (286 Da), at *m/z* 221 ([caffeoyl glucose-hydroxybenzyl alcohol-120 Da-$H^+$]$^-$), at *m/z* 251 ([caffeoyl glucose-hydroxybenzyl alcohol-90 Da-$H^+$]$^-$) and at *m/z* 263 ([caffeoyl glucose-hydroxybenzyl alcohol-78 Da-$H^+$]$^-$) by the loss of the 2-hydroxybenzyl residue (106 Da) and 78 Da (Fig. 3 and Table 2). It produced the $MS^3$ base peak at *m/z* 179 ([caffeic acid-$H^+$]$^-$) by the loss of glucosyl residue; it produced secondary peaks at *m/z* 161 ([caffeic acid-$H_2O$-$H^+$]$^-$) by the loss of the glucosyl residue (162 Da), at *m/z* 221 ([caffeoyl glucose-120 Da-$H^+$]$^-$), at *m/z* 251 ([caffeoyl glucose-90 Da-$H^+$]$^-$) and at *m/z* 263 ([caffeoyl glucose-78 Da-$H^+$]$^-$) (Fig. 3 and Table 2). The $MS^2$ secondary peaks at *m/z* 323, 221, 203, 251, 263 suggested that caffeic acid (C-9″) was connected with the glucosyl residue by an ester linkage (C-6′) (Fig. 3 and Table 2). In a recent study Bourjot et al. isolated poliothrysoside, 2-*O*-(6-*O*-glucosyl benzoyl)-2,5-dihydroxybenzyl alcohol from *Flacourtia ramontchi* stem bark. Based on the above points this compound was tentatively assigned as 2-*O*-(6′-*O*-glucosyl caffeoyl)-2-hydroxybenzyl alcohol **33**.

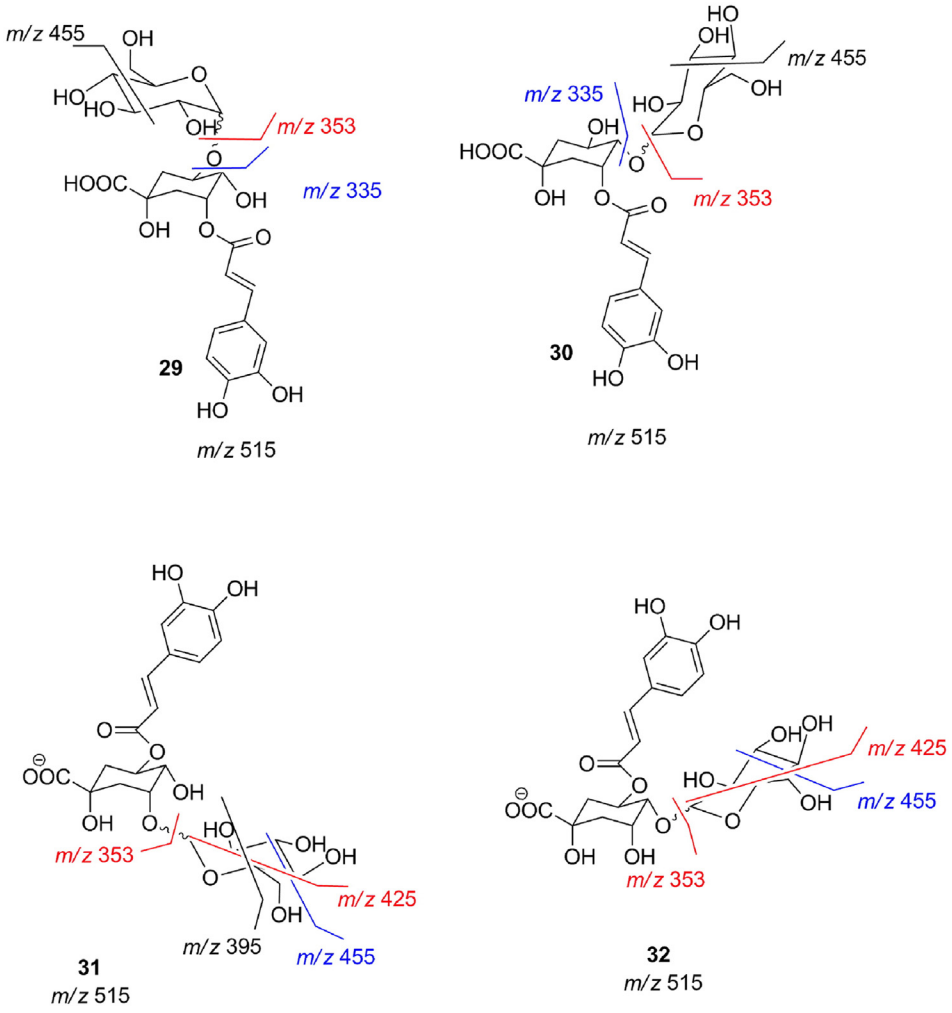


**Fig. 3.** Fragmentation pathways of 3-*O*-caffeoylquinic acid glucosides (**29** and **30**) and 5-*O*-caffeoylquinic acid glucosides (**31**–**32**) at *m/z* 515 in negative ion mode.

*Identification of gentisic acid glycoside (Mr 315) and salicylic acid glycoside (Mr 300)*

One peak was detected at *m/z* 315 which produced the $MS^2$ base peak at *m/z* 153 ([M- $H^+$ - 162 Da]—) by the loss of a glucosyl moiety (162 Da); it produced secondary peaks at *m/z* 245 by the loss of 70 Da and at *m/z* 109 ([gentisic acid-$CO_2$-$H^+$]$^-$) by the loss of $CO_2$ (Table 2). In the absence of authentic standards and literature this compound was tentatively assigned as gentisic acid glucoside.

One peak was detected at *m/z* 299 which produced the $MS^2$ base peak at *m/z* 137 ([M- $H^+$ - 162 Da]—) by the loss of a glucosyl moiety (162 Da) (Table 2); it produced secondary peaks at *m/z* 239, 209 and

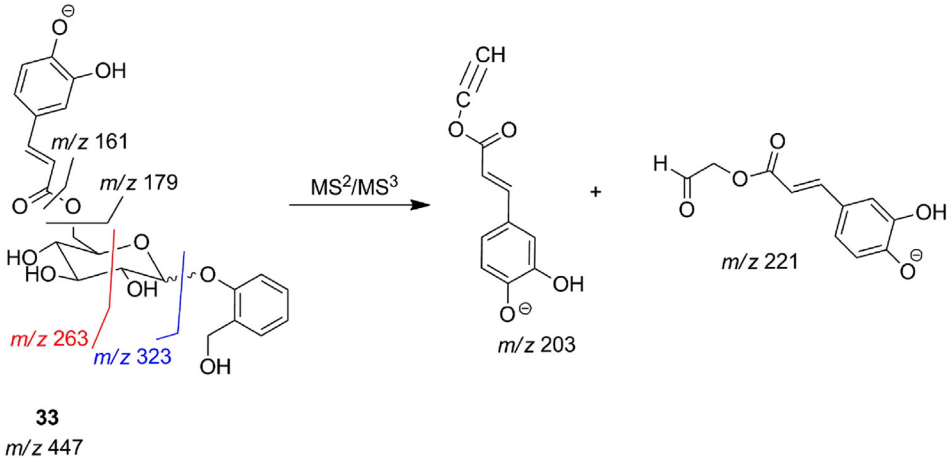


**Fig. 4.** Fragmentation pathways of 2-O-(6′-O-glucosyl caffeoyl)-2-hydroxybenzyl alcohol 33 at *m/z* 447 in negative ion mode.

179 ([glucose-$H^+$]$^-$) (Table 2). It produced the $MS^3$ base peak at *m/z* 93 ([salicylic acid-$CO_2$-$H^+$]$^-$) by the loss of $CO_2$ (Table 2). In the absence of authentic standards and literature this compound was tentatively assigned as salicylic acid glucoside.

## Conclusion

We have shown that *F. indica* and *F. inermis* fruits are rich sources of CGAs and derivatives reported in nature. Thirty-five phenolics were detected and characterized on the basis of their unique fragmentation pattern in the negative ion mode tandem MS spectra, most of them for the first time from these sources with four of them previously not reported in nature. The position of the glucosyl residue in CGA glucosides was assigned based on the hydrolysis experiments and their characteristic fragmentation at M-60, M-90, and M-120 in negative ion mode.

This is the first time when rare CGA glucosides are reported in nature and it is also possible to discriminate between them by high resolution and tandem LC-MS. In this study we are able to identify and characterize the caffeoyl glucoses and *p*-coumaroyl glucoses. These mass spectrometric methods can be used for the identification and characterization of novel isomeric CGA glucosides, caffeoyl glucoses, feruloyl glucoses, *p*-coumaroyl glucoses, sinapoyl glucoses, galloyl glucoses, dihydroxybenzoyl glycosides and hydroxybenzoyl glycosides present in fruits, vegetables, foods and beverages.

## Acknowledgment

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.foodres.2014.03.036.

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