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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.

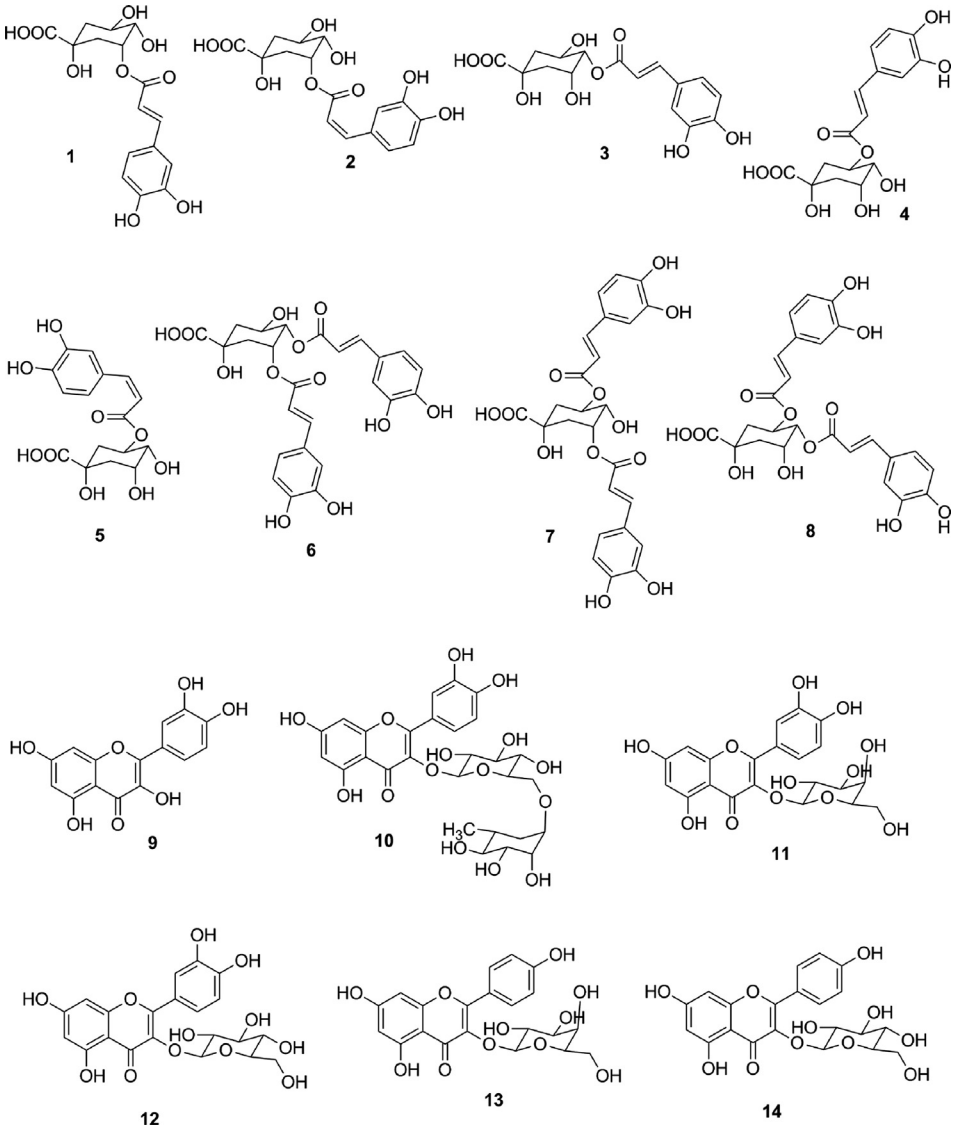

Fig. 1. Representative structures of *F. indica* and *F. inermis* fruits phenolics.