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Metabolic fingerprinting of banana passion fruits and its correlation with quorum quenching activity

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ABSTRACT

Banana passion fruit of the *Passiflora* genus, are commercially cultivated on a small to medium scale, mainly as edible fruits or as components of traditional herbal medicines. This subgenus comprises several species and hybrid specimens that grow readily in the wild. Due to their taxonomical complexity, many of these species have recently been reclassified (Ocampo Pérez and Coppens d'Eeckenbrugge, 2017), and their chemical profile has still to be determined. In this study, an ^{1}H NMR-based platform was applied to the chemical profiling of seven wild species of the *Passiflora* subgenus, and UHPLC-DAD-MS was additionally used for the identification of phenolic compounds. A total of 59 compounds were detected including 26 *O*- and *C*-glycosidated flavonoids and polyphenols, nine organic acids, seven amino acids, GABA, sucrose, glucose, myo-inositol, and five other non-identified compounds. Two of the identified compounds are the previously undescribed C-glycosyl flavonoids, apigenin-4′-*O*-*β*-glucopyranosyl, 8-*C*-*β*-(6″acetyl)-glucopyranoside and apigenin-4-*O*-β-glucopyranosyl-8-C-*β*-neohesperidoside. These *C*-glycosyl flavonoids were isolated to confirm their proposed structures by NMR and LCMS analysis. The PCA score plots obtained from the ^{1}H NMR data of the studied *Passiflora* samples showed *P. cumbalensis* and *P. uribei* as the species with the most distinguishable chemical profile. In addition, a correlation analysis using OPLS-DA was conducted between ^{1}H-NMR data and the quorum quenching activity (QQ) of *Chromobacterium violaceum* ATCC 31532. This analysis revealed *P. lehmannii*, and *P. uribei* extracts to be the most active, and apigenin-4′-*O*-*β*-glucopyranosyl, 8-*C*-*β*-(6″acetyl)-glucopyranoside and apigenin-4-*O*-β-glucopyranosyl-8-C-*β*-neohesperidoside were identified as possibly responsible for the QQ activity. To confirm this, QQ activity of both compounds was tested against *C. violaceum* ATCC 3153. An inhibition of violacein production of 0.135 mM (100 μg/mL) and 0.472 mM (300 μg/mL) was observed for apigenin-4′-*O*-*β*-glucopyranosyl,8-*C*-*β*-(6″acetyl)-glucopyranoside and apigenin-4-*O*-β-glucopyranosyl-8-C-*β*-neohesperidoside respectively, while bacterial growth was unaffected in both cases. Furthermore, both compounds showed the ability to inhibit the production of the toxoflavin of the phytopathogen *Burkholderia glumae* ATCC 33617.

1. Introduction

Passiflora species are highly appreciated and cultivated for their edible fruit, attractive flowers, and medicinal properties as mild tranquilizers and sedatives, for the treatment of insomnia, hysteria and epilepsy, and antidiabetic activity (Ingale and Hivrale, 2010). There are a number of reviews covering this wide range of bioactivities (Gadioli et al., 2018; Ingale and Hivrale, 2010) as well as their biotechnological applications (Corrêa et al., 2016). The northern Andes of Colombia and Ecuador are at the center of the *Passiflora* genus diversity (Ocampo Pérez et al., 2007), but most species have not been chemically characterized. Among them, banana passion fruits (oblong or ovoid berry, banana shape, with coriaceous or soft pericarp, light green color and that turns yellow when ripe), which belong to the subgenus *Tacsonia* of *Passiflora* genu, a low-level classification based on phenetic features (Ocampo Pérez and Coppens d'Eeckenbrugge, 2017). They are currently viewed with interest as new edible fruits source, though their large-scale commercialization is limited due to the restriction of their

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growth to certain areas of the Andes in S. America. However, given the existing great diversity of the genus, involving more than 50 species that grow above 2000 m of altitude and yield fruits with a good reputation in local and overseas markets, their agricultural exploitation could be feasible. This possibility could have an additional socio-economic impact on this region since it could constitute an interesting alternative as crops for small growers in this poverty and violence-stricken region of the high Andes, providing some relief for their dire situation (Ocampo Pérez and Coppens d'Eeckenbrugge, 2017). For this, it is essential to study and profile the major species, providing a basis for the selection of the most suitable.

There are many commercially interesting *Passiflora* species such as *P. mollissima* (Kunth) L.H. Bailey (syn. *P. tripartita* var. *Mollissima* Holm-Niels. & P. Jørg, Curuba de Castilla), *P. tarminiana* Coppens & V.E. Barney (Curuba india), *P. cumbalensis* (Karst) Harms (the rosy passion fruit), *P. pinnatistipula* Cav. (tintin) and *P. mixta* L.F. (Curuba de indio or Curuba de monte). Among them, *P. mollissima* is one of the most appreciated by consumers because of the organoleptic properties of its fruits. Its hydroalcoholic leaf extract has already been approved in Colombia as a mild tranquilizer (Ministerio de la Protección Social de Colombia, 2008). On the other hand, *P. tarminiana* is preferred as a crop by farmers because of its resistance to anthracnose (*Colletotrichum gloeosporioides*) (Primot et al., 2005).

Besides their nutritional value as fruit, *Passiflora* species are also known to have many bioactivities, revealing a potential as functional foods. Recently, antimicrobial properties related to quorum quenching activity has been reported for the *C*-flavonoids present in certain *Passiflora* species (Brango-Vanegas et al., 2014). Bacteria use quorum sensing (QS) systems to coordinate and synchronize their behavior by regulating a number of processes related to their metabolism as a reaction to different circumstances (Helman and Chernin, 2015). For example, it has been reported that toxoflavin production in *Burkholderia* spp. Is controlled by a QS system, the production of this phytotoxin being a key pathogenicity factor in rice and rot wilt (J. Kim et al., 2004). The expression of some virulence-related traits depends on QS and particularly many plant-pathogenic bacteria, rely on such systems for their survival in host plants (Barnard et al., 2007).

The chemistry of *Passiflora* plants has been the target of a considerable number of studies, but given the complexity posed by the bio- and chemo-diversity of banana *Passiflora* these have only been studied partially. In some of the species, flavonoids and triterpenoidal saponins have been found to be the main metabolites and amino acids, while alkaloids and cyanogenic glycosides have also been detected but as minor components (Dhawan et al., 2004; Gadioli et al., 2018; Ingale and Hivrale, 2010). Among the flavonoids present in *Passiflora*, *C*-glycosyl type flavonoids are well-known for their strong and diverse bioactivities including antitumoral, hepatoprotective, anti-inflammatory, anti-diabetes, antiviral as well as antibacterial and antifungal activities (Ingale and Hivrale, 2010). They are also known to produce quorum sensing disruption (Brango-Vanegas et al., 2014). The detected saponins, on the other hand, are thought to be associated to natural defense systems in the plant, protecting them from the attack of potential pathogens (Osbourn, 2003) and contributing to their insecticidal, antibiotic, and fungicidal activities. *Passiflora* saponins have also been reported to have minor antiinflammatory, hypocholesterolemic, xanthine oxidase-inhibiting, antifungal, antiprotozoal, antinociceptive and antitumoural properties (Sparg et al., 2004).

Several *Passiflora* species have been studied using the conventional bioactivity-guided fractionation approach searching for the specific metabolites that could be related to these alleged bioactivities. Unfortunately, as in many cases, this time-consuming design yielded only a few compounds, in general with less activity than expected according to that exhibited by the plants. These disappointing results, echoed over most natural product studies, decreased the expectations of these products as a potential source of new drugs or leads, posing the need for the development of a more efficient experimental design in terms of its outcome.

A few years ago, such an option appeared in the shape of a new approach based on the idea of acquiring and comparing the whole metabolic profiles of plants, that are considered to reflect mechanisms underlying alleged bioactivities. This approach, which uses bio-chemometric tools was conceived within the systems biology framework, is known as metabolomics and aims at the acquisition and comparison of profiles of the low molecular mass metabolites of plants and other organisms and their correlation with proposed bioactivities, ultimately to select those metabolites which could be associated to them (Inui et al., 2012). The goal of research has thus moved from the often indiscriminate isolation of compounds to the multivariate comparison of metabolic profiles of active and inactive samples (Wu et al., 2016). There are many examples of the efficient application of this approach to the discovery of bioactive compounds. The prioritization of 46 sponge-associated actinomycetes was done using metabolomic analysis by LC-HRMS and NMR to identify their distinct chemical profiles, as well as their anti-trypanosomal activity against *Trypanosoma brucei* TC221showing a half maximal inhibitory concentration (IC50) of $<$ 20 μg/mL (Cheng et al., 2015). In another case, the use of NMR-based metabolomics resulted in the discovery of a prenylated isatin antibiotic produced by *Streptomyces* sp. MBT28, which was then identified as 7-prenylisatin using NMR-based metabolomics (Wu et al., 2015). These studies, among many others, are evidence of the efficiency of metabolomics in facilitating the discovery of unreported bioactive compounds.

Different analytical platforms have been used in metabolomics, the most common being LC-MS and ^{1}H NMR. The advantage of the latter is its high reproducibility and ease of quantification as signal intensity is directly related to the molar concentrations (Smolinska et al., 2012). Additionally, it allows an unbiased analytical approach regarding the chemical structure of compounds to be detected and is characterized by very simple and fast sample preparation methods and high-speed throughput.

The aim of this study was the comparative analysis of the metabolic profiles of *Passiflora* species collected in the Colombian Andes and the correlation of their chemical profiles with their inhibitory quorum sensing activity. For this, a metabolomics study of the six most representative species from the subgenus of banana passion fruits was conducted. Our strategy included a non-targeted approach using NMR analysis to identify the major compounds (Kim et al., 2010) and LCMS to confirm the identity of *C*-flavonoids (Farag et al., 2016). The quorum quenching activity of crude extracts of *Passiflora* leaves was tested with the *Chomobacterium violaceum* ATCC 31532 biosensor, and this information was correlated with the metabolic profiles by means of multivariate data analysis.

2. Results and discussion

Samples of *Passiflora* species were collected in the central and southern regions of the Colombian Andes. Wild types were used instead of cultivated samples which generally are hybrids (Table 1). All samples were identified on the basis of their morphological characteristics by the taxonomist Gustavo Morales and voucher samples were deposited in the collection of the Instituto de Ciencias Naturales – Universidad Nacional de Colombia as reported in Table 1.

In the first stage, an untargeted analysis using ^{1}H NMR chemical profiling was performed to obtain specific fingerprints of the *Passiflora* selected species and their related compounds. The Leiden Natural Products Laboratory database, the Chenomx NMR Suite 8.2 (Chenomx Inc., Edmonton, Alberta, Canada) database and the Human Metabolome Database (HMDB) were used for the identification of compounds in the extracts. This was complemented by the LCMS analysis of butanol fractions of the plant extracts that provided further information on the phenolic compounds. Correlations between the chemical composition of the plants and their quorum quenching activity were established submitting all this data to MVDA including principal component

Table 1
Banana passion fruits samples collection data.

Species of *Passiflora* collected	Number of voucher ICN collection[a]	Subgenera (Section/series)	Number of samples
Passiflora mollissima (Kunth) L.H.Bailey *Passiflora tripartita var mollisima* (Kunth) Holm-Niels & P. Jorg (synonym). (The Plant List, 2013a)	COL599223	*Tacsonia* (*Bracteogama*)	7
Passiflora tripartita (Juss.) Poir *Passiflora tripartita var tripartita* (Kunth) Holm-Niels & P. Jorg. (synonym) (The Plant List, 2013b)	COL599245	*Tacsonia* (*Bracteogama*)	1
Passiflora cumbalensis (H. Karst) Harms	COL599225	*Tacsonia* (*Bracteogama*)	10
Passiflora tarminiana Coppens & V.E. Barney	COL599247	*Tacsonia* (*Bracteogama*)	13
Passiflora mixta L.F.	COL599246	*Tacsonia* (*Tacsonia*)	12
Passiflora pinnatistipula Cav.	COL599224	*Tacsonia* (*Colombiana/Poggendorffia*)	3
Passiflora uribei L. K. Escobar		*Tacsonia* (*Colombiana/ Fimbriatistipula*)	3
Passiflora lehmanni Mast	COL599222	*Passiflora* (*Tiliaefoliea*)	2

[a] GPS information for collection sites is given in Table S3.

analysis (PCA), and orthogonal partial least squares (OPLS) analyses.

2.1. NMR metabolic fingerprint of Passiflora species

Freeze-dried samples of *Passiflora* leaves were extracted with CH_3OH-d_4-KH_2PO_4 buffer in D_2O (1:1, v/v), in order to obtain a wide range of metabolites that included sugars, amino acids, saponins and flavonoids, as these have been reported to be the major compounds in *Passiflora* species. The resulting spectra of the *Passiflora* extracts were analyzed with the Chenomx™ database, along with our in-house database and literature data. Because of the intense overlapping of proton signals, the identity of the proposed compounds was verified by 2D-NMR experiments (J-resolved; ^1H–^1H-correlated spectroscopy-COSY- and heteronuclear multiple bond correlation -HMBC-). The complete NMR data for the identified compounds is presented in the supplementary information (Supp. Table 1, and Supp. Fig. 6–16).

The ^1H-NMR spectra of the *Passiflora* extracts proved to be very similar. As can be observed with the example of the *P. tarminiana* extract, the presence of amino acids, carbohydrates, and flavonoids was confirmed (Fig. 1 and in Figure SI-1 supporting information for the other extracts). The main differences between species were observed in the aromatic region, suggesting that the composition of the flavonoids and other phenolics was distinctive between species (Fig. 2).

The analysis of the extracts revealed the presence of nine organic acids, seven amino acids, GABA, sucrose, glucose, myo-inositol and five other unidentified compounds. Their distribution among the studied species is presented as a barcoding of primary metabolites in Fig. 3, and shows that the species exhibiting most diversity were *P. tarminiana, P. cumbalensis, P. mollissima and P. tripartita* (Juss.) Poir (syn. *P. tripartita* var. *Tripartita*), while the least complex were those of *P. uribei* L. K. Escobar and *P. lehmannii* Mast. The latter was included in this study as an outlier in order to compare the chemical composition of *Passiflora* spp. In two different subgenera. The content of sugars, polyhydroxyalcohols and other compounds was found to be very similar for the seven extracts, but the content of organic acids and amino acids did not show a distinct distribution pattern.

The amino acid content of two species of the *Passiflora* genus has been reported. Twenty one amino acids have been identified in *P. incarnata* (Gavasheli et al., 1974) and 17 in *P. edulis* seeds, an ingredient of "Tainung No. 1", a passion fruit formulation used in China (Liu et al., 2008). The presence of γ-aminobutyric acid (GABA) was detected in most of the studied species, including *P. mollisima,* the species that is approved in Colombia as a mild tranquilizer (Ministerio de la Protección Social de Colombia, 2008) and in *P. uribei,* that appears to be the most abundant source of this compound among the studied samples. It is thought that GABA, that has also been detected in *P. incarnata*, might be responsible for the anxiolytic and sedative properties of Passion fruit leaf extracts (Elsas et al., 2010), though the extent of its pharmacological significance is still unclear (Elsas et al., 2010; Jawna-Zboińska et al., 2016). Trigonelline, which was identified in all samples as a minor compound has been associated to neuroprotective, anti-migraine, sedative, memory-boosting and hypoglycemic activities (Zhou et al., 2012). All these pharmacological properties have been detected in different *Passiflora* spp. Extracts and support its traditional medicinal use. The presence of 5-carboxymethyl-2,5-dihydrofuran-2-one was unexpected as this compound has only been previously isolated from an unrelated organism, the marine sponge *Xestospongia* sp. Collected in the island of Viti Levu (Fiji). This compound has been reported to possess a mild cytotoxic activity against P388 murine leukemia cells (Quinoa et al., 1986), and has been identified as a key intermediate in the catechol branch of the β-ketoadipate pathway for the degradation of many arenes by a variety of organisms including microorganisms (Ribbons and Sutherland, 1994). The microbial origin of this compound can explain the variability in its concentration in some of the examined samples, including those of *P. caerulea* and *P. incarnata* acquired in The Netherlands (results not shown). Finally, considering that saponins have been reported as major compounds in other species of the subgenus *Passiflora* of the *Passiflora* genus, i.e., *P. edulis var flavicarpa* (Serie *Incarnatae*) (Yoshikawa et al., 2000), *P. alata* (Serie *Quadrangulares*) (Reginatto et al., 2004), *P. quadrangularis* (Serie *Quadrangulares*) and *P. ligularis* (Serie *Tiliaefoliae*), it is noteworthy that no saponins were detected with these methods in the studied species (unpublished results).

The direct analysis of the content of phenolic compounds in the NMR spectra of the extracts was hindered by the high complexity of the aromatic region, the shifting of ^1H NMR signals and the low concentration of some of these compounds. Thus, the main phenolics, including some *C*-glycosyl flavonoids and catechins, had to be isolated from the extracts for their identification. Their chemical shifts in CH_3OH-d_4 in buffer (90 mM KH_2PO_4 in D_2O) solvent are presented in Supp. Table 2.

The signals for a *C*-neohesperidoside glycosyl were detected in most of the ^1H-NMR spectra of the *Passiflora* extracts, except in *P. mollissima* and *P. mixta* samples that showed a very low amount if any. The identification of the *C*-neohesperidoside diglycoside was based on the signals for methyl groups at 0.60 ppm that were assigned to its rhamnose methyl protons. This shift is due to the spatial shielding effect of the A-ring of the flavonoid aglycone when the disaccharide moiety is attached at position C-6 or C-8. The rotational barrier around the *C*-glycosidic linkage also leads to signal doubling in the NMR spectra, as a result of the presence of two main conformers (Camargo et al., 2012; Larionova et al., 2010).

The LC-MS analysis of flavonoids using MS and MS/MS data proved to be useful for the structural elucidation of both *O*-glycosides and *C*-glycoside flavonoids. This technique has been widely used for flavonoid

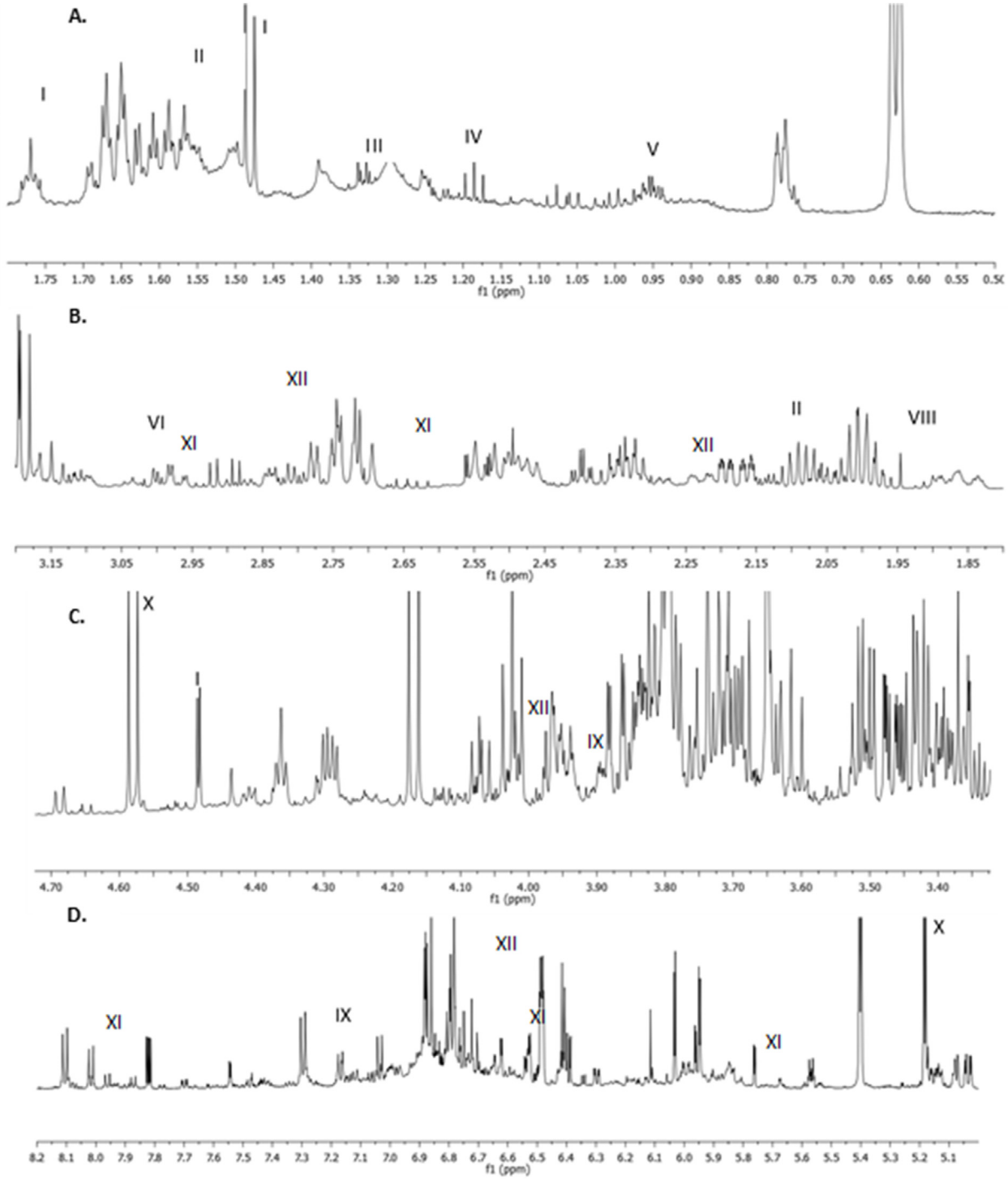

Fig. 1. ^{1}H NMR spectrum of *Passiflora* leave extract showing aliphatic, sugar and aromatic region and some assignments: ascorbic acid (I), proline (II), threonine (III), ethanol (IV), leucine (V) pipecolic acid (VI) and acetic acid (VIII) (A and B). Sugar region ascorbic acid (I) and glucose (X) (B and C). Phenolic region glucose (X), tyrosine (IX), 5-carboxymethyl-2,5-dihydrofuran-2-one (XI) shikimic acid (XII) (D). The whole NMR signals assignation can be consulted at Table 1 supporting information.

characterization in *Passiflora* extracts (Farag et al., 2016; Simirgiotis et al., 2013; Zucolotto et al., 2012) In all the studied samples, the BuOH fractions were analyzed by reversed-phase UHPLC-DAD/ESI-2 QToF-MS. Peaks were identified by comparison of retention times with those of external standards, mass spectra and UV analysis. The presence of *O*- or *C*- glycosylation, hexoses, pentoses, and acetyl groups were assigned by the MS/MS data analysis of well-established fragmentation patterns such as $[M\text{-}162]^{+/-}$ (hexoses), $[M\text{-}132]^{+/-}$ (pentoses), $[M\text{-}18]^{+/-}$ and $[M\text{-}120/90]^{+/-}$ cross-ring cleavages $[(O\text{–}C1 \text{ and } C2\text{–}C3)]^{\cdot}$ or $[(O\text{–}C1 \text{ and } C3\text{–}C4)]$ for *C*-hexosides, $[M\text{-}90/60]^{+/-}$ for *C*-pentosides, and [M-104/74] for *C*-deoxyhexosides, among other ions, used for flavonoid characterization (Figueirinha et al., 2008). This MS-based approach is useful for positional isomer identification. For example, the differentiation between luteolin-6-*C*-glucoside (isoorientin, **14**) and luteolin-8-*C*-glucoside (orientin, **16**) is based on the high abundance of the product ion at *m/z* 429 $[M\text{-}18\text{-}H]^{-}$ in 6-*C*-hexoside, which is less intense in 8-*C*-glucoside (Farag et al., 2016).

In total, 34 phenolics were identified. Supp Table 2 includes the

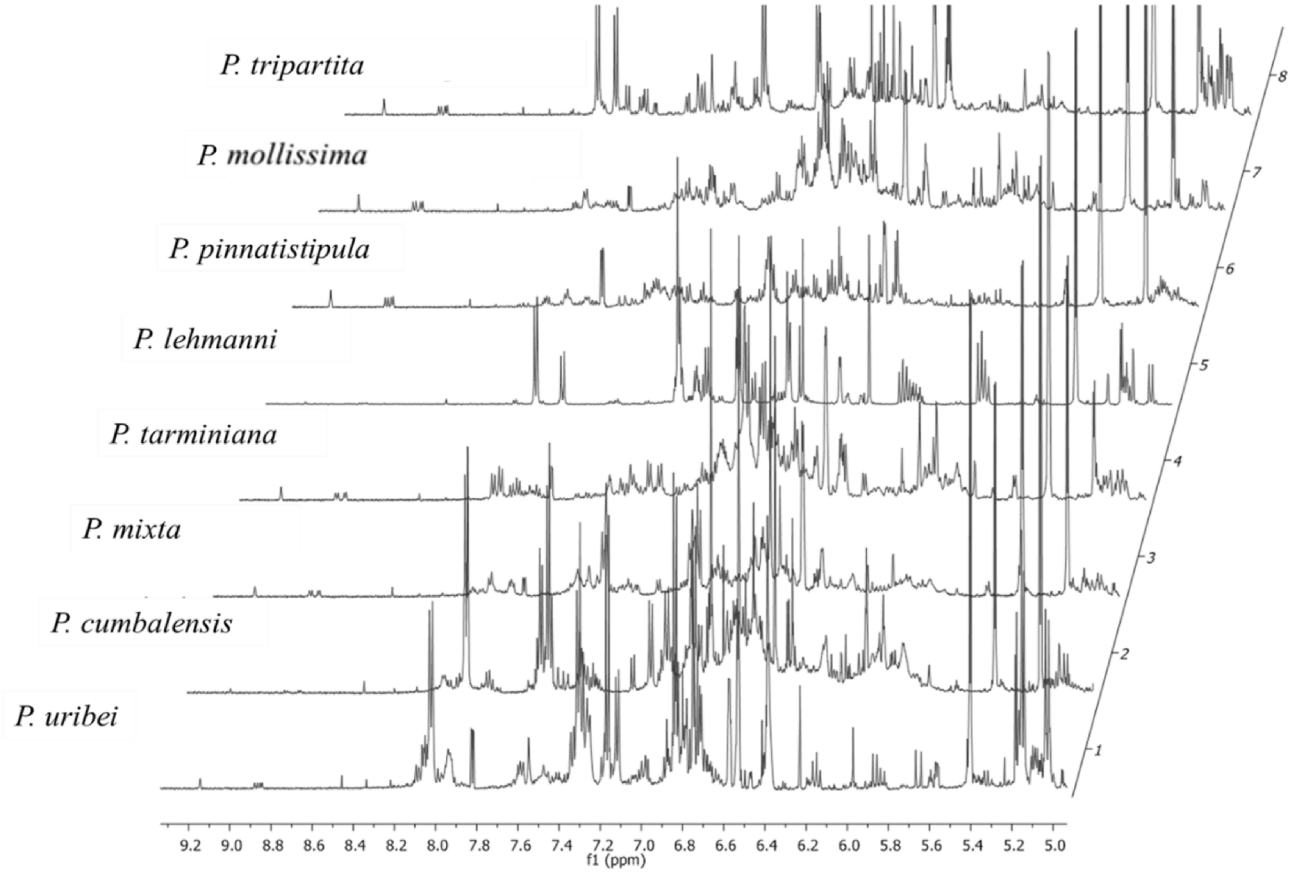

Fig. 2. The main differences for *Passiflora* samples can be observed in the aromatic region, suggesting a different composition of flavonoids and other polyphenolic compounds for each species.

NMR data (CH_3OH-d_4 in buffer 90 mM KH_2PO_4 in D_2O) and retention times, I_{max}, and experimental *m/z* and MS/MS data obtained by HRMS-ESI(−). Information on NMR data measured in other solvents such as MeOD and DMSO is recorded in Supp. Table 3.

The LC-MS analysis of the BuOH fraction of *Passiflora* species (Fig. 4) revealed a wide metabolic diversity in some of the species as shown by the profiles of *P. tarminiana*, *P. mixta, P. tripartita* and *P. mollissima,* as well as some less complex profiles such as those of *P. uribei* and *P. lehmannii* extracts, in agreement with their NMR profiles. Interestingly, the profiles of *P. tripartita* and *P. mollissima* showed some significant differences while the profile of *P. mollisima* was similar to that of *P. tarminiana* (Fig. 4).

The flavonoids identified in the studied samples (Supp. Table 2) included luteolin-derivatives (**10, 14–16, 20, 22–24, 28, 30, 34**) apigenin-derivatives (**1, 2, 9, 11–13, 17–19, 21, 31**) and chrysin **(25, 27, 33**) aglycones, along with some catechins (**3–5, 8**) and procyanidins (**6, 7**). Luteolin derivatives were found to be dominant in *P. mollissima,* but less abundant in *P. uribei* and *P. mixta*. The compound 4′-methoxy-luteolin-8-*C*-6″acetylglucopyranoside (**34**) described previously by us (Ramos et al., 2010) has been proposed as a chemical marker for *P. mollissima* (Simirgiotis et al., 2013). However, it was found also in *P. mixta, P. tarminiana* and *P. uribei*. But not in *P. tripartita*.

Apigenin-related flavonoids have been selected as chemical markers for *P. alata* by the Brazilian Pharmacopoeia (Farmacopéia, 2010). However, in the studied *Passiflora* samples, these were detected in all species, except in *P. cumbalensis* extracts, in which chrysin *C*-glycosides were found instead as highly abundant compounds. These chrysin derivatives were also found in *P. tripartita,* and *P. mixta* extracts, but in small quantities. Chrysin had been previously isolated from *P. caerulea* and proposed as an anxiolytic compound (Wolfman et al., 1994). Catechin derivatives were detected in large amounts in *P. tarminiana* and in the two varieties of *P. tripartita*. Interestingly, catechins have also been reported to induce anxiolytic activity (Vignes et al., 2006). However, the biological activity of these particular catechins still has to be determined.

Quorum quenching active butanolic extracts of *P. lehmannii* and *P. uribei* yielded two previously unreported flavonoids, **1** and **2** respectively, as the major compounds. The (–)-HRESIMS spectra of flavonoid **1** of the *P. lehmannii* extract showed an ion at *m/z* 635.1624 $[M-H]^-$ suggesting a molecular formula of $C_{29}H_{32}O_{16}$. The MS/MS spectrum of the parent ion at *m/z* 635 yielded ions at *m/z* 473 $[M\text{-hexose}]^-$ and 413 $[M\text{-H-hexose-}CH_3COO]^-$. The ^{1}H-NMR spectrum (400 MHz, Methanol-d_4) (Supp Fig 6) of this compound showed characteristic signals of apigenin with a monohydroxilated aromatic B ring (δ_H 8.03, 2H, d, J = 8.4 Hz; δ_H 7.26, 2H, d, J = 8.4 Hz), a penta-substituted A ring (δ_H 6.28, 1H, bs) and the characteristic H-3 proton of the C ring (δ_H 6.68, 1H, bs), along with two *β*-anomeric protons (δ_H 5.04, 1H, d, J = 7.4 Hz and 4.99, 1H, d, J = 10.2 Hz). The analysis of the coupling constants showed that both sugar moieties correspond to β-glucose residues. Assignment of the glucose residues was supported by the HMBC correlation from the glucose protons H-1″ (δ_H 4.96) and H-2″ (δ_H 4.12), with the aromatic C-8 carbon at δ_C 104.6, suggesting a *C*-glycosidic bond in the A ring (Supp Fig 8). A similar analysis showed the HMBC correlation from the anomeric proton H-1‴ (δ_H 5.03), with the aromatic carbon C-4′ at ä$_C$ 157.4, suggesting an *O*-glycosidic bond to the B ring of the flavonoid moiety. The correlation from both H-6″ protons at δ_H 4.47 and 4.28, to the carbon assigned to the acetate carboxyl at δ_C 173.1 suggested the presence of an acetyl group on 6″ of the *C*-glucopyranoside residue (Fig. 5A). Thus, compound **1** was identified as the previously undescribed flavonoid apigenin-4′-*O*-*β*-glucopyranosyl,8-*C*-*β*-(6″acetyl)-glucopyranoside. The NMR data are summarized in Table 2.

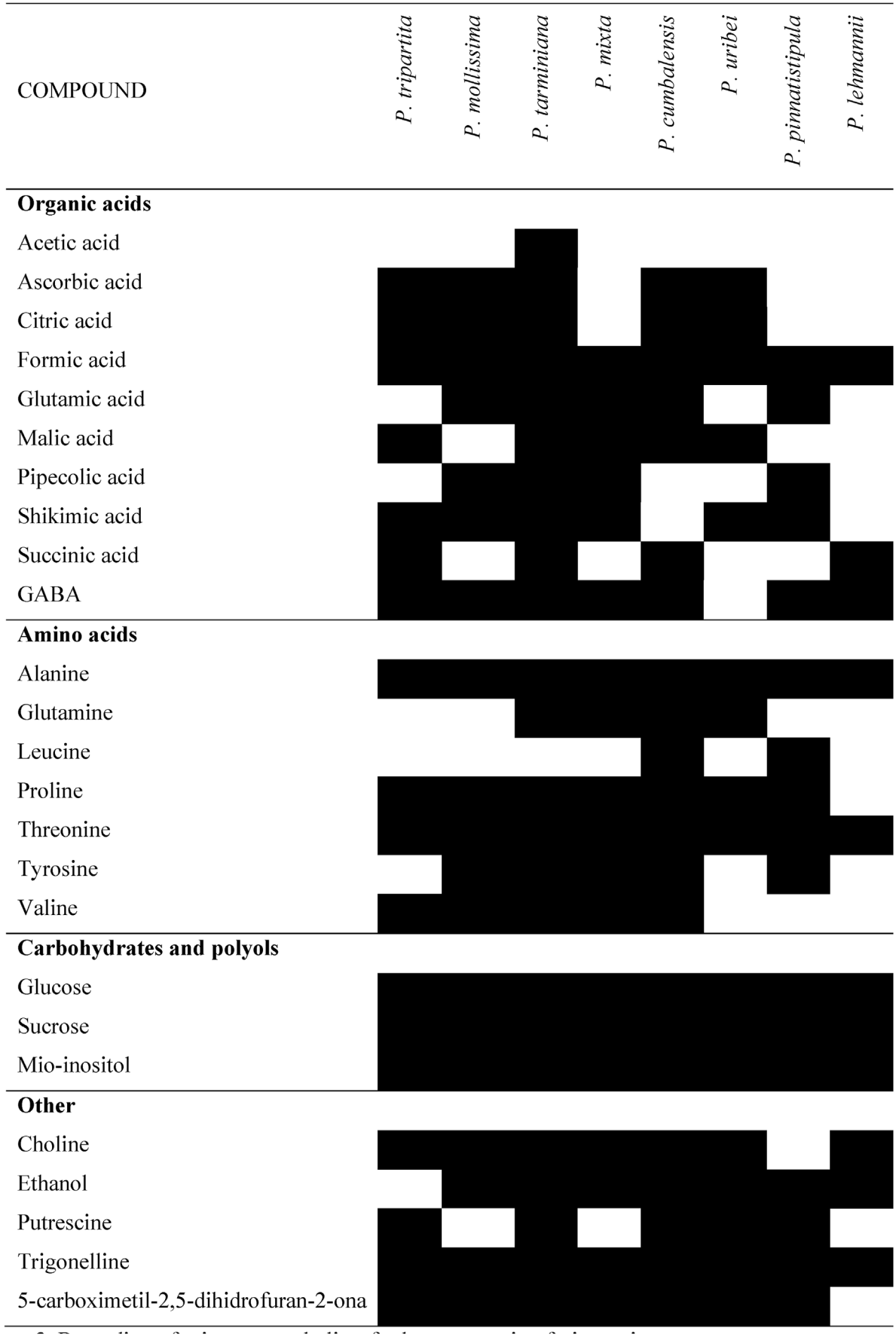

Fig. 3. Barcoding of primary metabolites for banana passion fruit species.

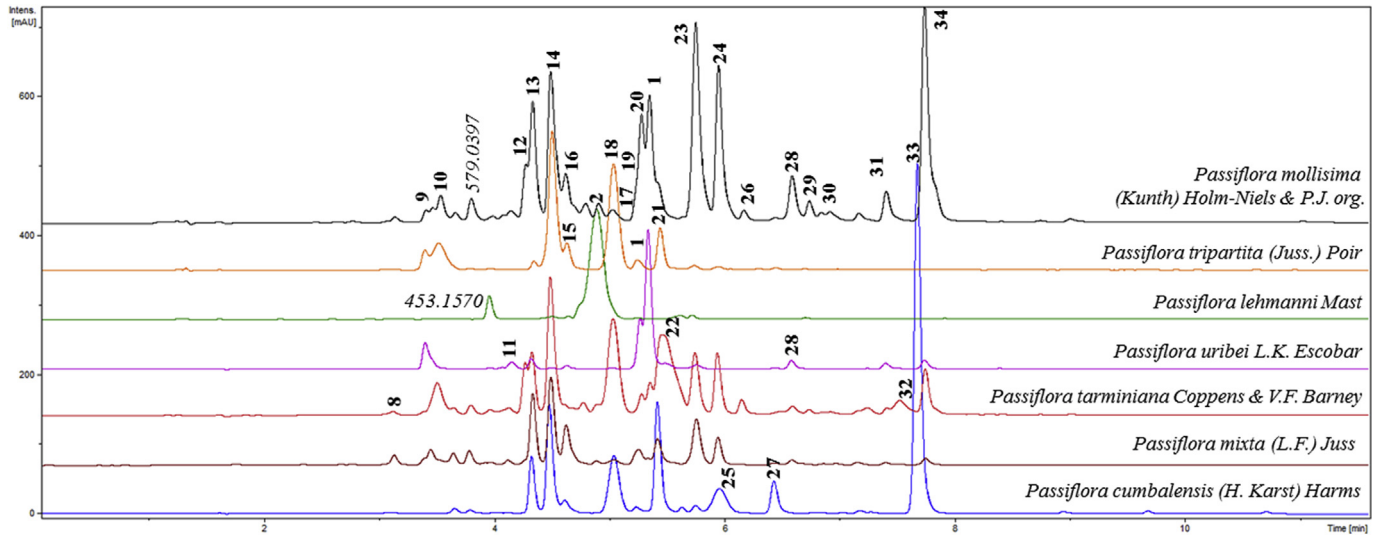

Fig. 4. UHPLC chromatograms (340 nm) of the butanolic extract of *Passiflora* species. Bold numbers correspond to identified compounds, and numbers in italics to the *m/z* of unidentified compounds.

The (–)-HRESIMS spectra of compound **2** yielded an ion at *m/z* 739.2091 $[M-H]^-$, corresponding to a possible molecular formula of $C_{33}H_{40}O_{19}$, that together with the ion at *m/z* 413 [M-hexose-deoxyhexose-H]$^-$ obtained with the MS/MS data of the parent ion, suggested the presence of a flavonoid bearing two hexoses and one deoxyhexose residue. The ^{1}H-NMR data (400 MHz, Methanol-d_4) (Supp Fig 11) for this compound, revealed signals that are characteristic of apigenin showing two main conformers with paired signals at δ_H 7.97 (d, J = 8.7 Hz) [7.81 (d, J = 8.5 Hz)]; 6.93 (d, J = 8.7 Hz) [6.94 (d, J = 8.5 Hz)]; 6.59 (s) [6.62]; 6.27 (s) [6.25 (s)], together with three anomeric protons (δ_H 5.15 (d, J = 1.3 Hz) [5.31 (d, J = 1.7 Hz)]; 5.03 (d, J = 9.9 Hz) [5.15 (d, J = 9.8 Hz)]; 4.39 (d, J = 7.7 Hz) [4.27(d, J = 7.9 Hz)] and a highly overlapping region for the carbinolic protons of the three sugar moieties. The presence of a neohesperidoside moiety was determined by the HMBC (Sup Fig. 11) correlation from the α-rhamnopyranosyl anomeric proton at δ_H 5.15 (d, J = 1.8 Hz) [5.31 (d, J = 1.7) Hz]/δ_C 102.0 [101.3] to the C-2" (δ_C 77.6 [76.7]) of glucopyranoside. The bonding of the neohesperidoside moiety to the carbon C-8 was revealed by the HMBC correlation of the β-anomeric glucoside proton at δ_H 5.03 (d, J = 9.9 Hz) [5.15 (d, J = 9.8 Hz)]/δ_C 73.7 [75.3], and H-2″ glucopyranose proton at δ_H 4.26 (dd, J = 9.9; 8.5) [4.07 (bt, J = 9,3)] to the C-8 carbon at δ_C 104.7. The presence of a shielded methyl group at δ_H 0.73 (d, J = 6.2) [0.88 (d, J = 6.2)] of the rhamnopyranosyl CH_3-6‴in a C-8 linked neohesperidoside moiety (α-rhamnopyranosyl-(1 → 2)-β-glucopyranoside), due to the strong diamagnetical shift caused by the anisotropic effects of one of the aromatic rings of the apigenin moiety in the preferred conformation of the compound (Larionova et al., 2010). Finally, the position of glycosylation was determined to be C-4′ by the HMBC correlation between the anomeric proton at δ_H 4.39 (d, J = 7.44) [4.27 (d, J = 7.9] and C-4′ at δ_C 162.7 as is shown in Fig. 5B. The complete assignment of NMR signals was done using COSY, HSQC and *J*-resolved spectra and are summarized in Table 2 (see supporting information). Compound **2** was thus identified as apigenin-4-*O*-*β*-glucopyranosyl-8-*C*-*β*-neohesperidoside. The presence of this compound in *P. coactilis* had been proposed by Escobar et al., using enzymatic hydrolysis, TLC co-chromatography, 100 MHz NMR and UV analysis for its identification (Escobar et al., 1983). We have now completed this identification with complete NMR and MS data.

2.2. Taxonomical relationships from the metabolic fingerprint of samples from Passiflora species

Previous metabolomic studies of *Passiflora* species showed the presence of *C*- and *O*-glycosil flavonoids, mandelonitrile glycosides and fatty acid conjugates (Farag et al., 2016; Otify et al., 2015). The barcoding (supporting information Fig. 5) showed that major differences between different species are mostly due to the flavonoid content as well as amino acids.

The PCA-Pareto scaling analysis for the *Passiflora* extracts yielded four main groups, as depicted by the score plot of PC1 (26.7%) and PC2 (18.1%) R^2 0.652, Q^2 0.421 (Fig. 6). The only sample that does not belong to the *Tacsonia* subgenera, *P. lehmannii* (which belongs to *Passiflora* subgenera), which was included here as an outlier, unsurprisingly proved to be one of the less related species among the evaluated samples. A highly differentiating pattern was also observed for *P. uribei*

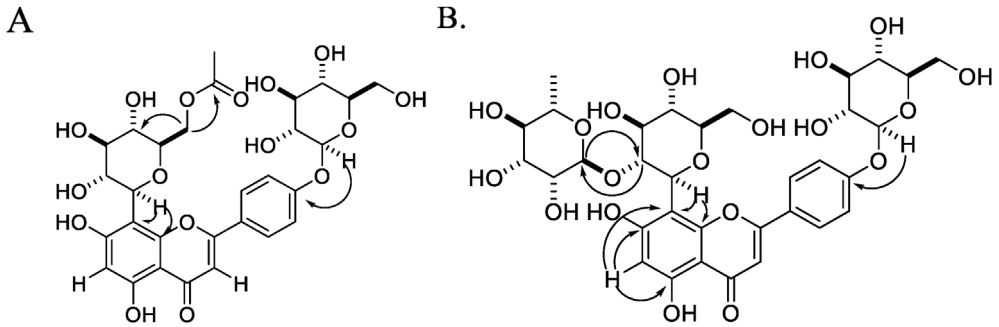

Fig. 5. Unreported flavonoids identified as major components from *Passiflora lehmannii* Apigenin-4′-*O*-*β*-glucopyranosyl, 8-*C*-*β*-(6″acetyl)-glucopyranoside (1) (A) and *Passiflora uribei* Apigenin-4-*O*-β-glucopyranosyl-8-C-*β*-neohesperidoside (2) (B). Arrows represent key HMBC correlations.

Table 2
^{1}H and ^{13}C NMR data for Apigenin-4-*O*-glucopyranosyl, 8-*C*-(6″acetyl)- β-glucopyranoside (**1**) and Apigenin-4-*O*-β-glucopyranosyl-8-C-*β*-neohesperidoside (**2**) (Methanol-d_4, δ in ppm, *J* in Hz).

Position	Compound 1		Compound 1	
	δ_H (mult, *J* value in Hz)	δ_C (ppm)	δ_H (mult, *J* value in Hz)	δ_C (ppm)
2		165.5		166.6 [165.9]
3	6.68 (bs)	104.8	6.59 (s) [6.62 (s)]	102.0 [103.6]
4		184.1		184.1 [184.4]
5		161.1		162.7 [162.9]
6	6.31 (s)	101.8	6.27 (s) [6.25 (s)]	99.8 [101.3]
7		162.8		164.1 [164.3]
8		104.6		105.3 [105.3]
9		157.4		157.8 [156.4]
10		104.8		106.0 [105.8]
1′		126.4		123.5 [123.4]
2′-6′	7.24 (d, 8.4)	118.1	7.97 (d, 8.7) [7.81 (d, 8.5)]	130.1 [129.6]
3′-5′	8.03 (d, 8.4)	129.8	6.93 (d, 8.7) [6.94 (d, 8.5)]	117.0 [117.1]
4′		161.8		162.7 [162.7]
C-8 glucose				
1″	4.96 (d, 9.3)	75.7	5.03 (d, 9.9) [5.15 (d, 9.8)]	73.7 [75.3]
2″	4.12 (t, 9.3)	73.0	4.26 (dd, 9.9; 8.5) [4.07 (bt, 9,3)]	77.6 [76.7]
3″	3.57 (dd, 9.3, 7.9)	80.2	3.68 (dd, 8.5; 5.3) [3.73 (bt, 8.7)]	81.6 [81.2]
4″	3.70 (m)	72.5	3.65 (m)	72.4 [72.4]
5″	3.67 (m)	80.4	3.48 (m) [3.71]	82.8 [82.7]
6″	4.47 (dd, 12.0, 2.0)	65.2	3.97 (m)	62.6 [62.3]
	4.28 (dd, 12.0; 4.7)		3.80 (m)	
CH_3CO		173.1		
CH_3CO	1.89 (bs)	21.0		
4-O-glucose				
1‴	5.03 (d, 7.4)	101.6	4.39 (d, 7.7) [4.27 (d, 7.9)]	105.6 [105.2]
2‴	3.48 (dd, 9.6, 7.4)	74.9	3.17 (m) [3.12 (m)]	75.9 [75.9]
3‴	3.40 (bt, 9.4)	71.3	3.34 (m) [3.34 (m)]	78.2 [78.1]
4‴	3.43 (m)	71.3	3.32 (m)	71.3 [71.7]
5‴	3.56(m) overlaped	79.8	3.32 (m)	82.8 [82.7]
6‴	3.73 (dd, 12.0, 5.6)	62.5	3.79 (m)/3.68 (m) [3.79 (m)/3.66 (m)]	62.6 [62.3]
	3.92 (dd, 12.0, 2.00)			
Rhamnose				
1‴			5.13 (d, 1.8) [5.31 (d, 1.7)]	102.0 [101.3]
2‴			3.88 (dd, 3.1, 1.8) [3.71 (dd, 3.1, 1.7)]	72.0 [72.0]
3‴			3.59 (dd, 9.6, 3.1) [3.24 (dd, 9.4,3.1)]	72.0 [71.7]
4‴			3.31 (bt, 9.6) [3.22 (bt, 9.4)]	83.2 [82.9]
5‴			2.55 (dq, 9.6, 6.2) [2.11 (m)]	68.3 [68.1]
6‴			0.73 (d, 6.2) [0.88 (d, 6.2)]	18.1 [18.1]

[a] In square bracket the values for less abundant conformer. Abundance 1.00: 0.45 according to ^{1}H NMR.

samples. On the other hand, one of the groups included samples of *P. mixta*, *P. mollissima* and *P. tarminiana* that are species known to hybridize easily, although each one recovers its own morphology and genetic distinctiveness after very few generations, suggesting that they are very close in evolutive terms (Ocampo Pérez and Coppens d'Eeckenbrugge, 2017). This behavior is in agreement with the data obtained from the PCA. The close similarity between *P. mollissima* and *P. tarminiana* suggests that the former could be used alternatively for medicinal purposes as a mild sedative drug. The *P. cumbalensis* samples were grouped apart from others, revealing a slight difference with other *Passiflora* species as previously reported (Ocampo Pérez and Coppens d'Eeckenbrugge, 2017). This differentiating pattern could be explained by the high contents of chrysin derivatives detected in this study for *P. cumbalensis* (Supp. Table 2 and Fig. 4 and supporting info Fig. 21).

2.3. Bioprospecting studies of Passiflora species related to QQ activity

Having completed the metabolic profiling of the studied *Passiflora* species, the next step was to relate those profiles with the biological activity observed for the extracts in order to identify the compounds responsible for such an activity (Wu et al., 2015). The selected bioactivity was quorum sensing inhibitory activity (QSI activity) because the search of anti-pathogenic compounds seemed to be a better strategy than the search for antibiotics, in terms of reducing the damage in the host, without generating induced resistance in the pathogen. Several small molecules including *C*-glycoside flavonoids, vanillin, 3-indolyacetonitrile, among others have been reported to be quorum sensing inhibitors (Grandclément et al., 2016), (Brango-Vanegas et al., 2014).

The MeOH/H_2O extracts of *Passiflora* species were tested for the inhibition of violacein production using *Chromobacterium violaceum* ATCC 31532 as a biosensor (supporting info table 4). Results showed that *P. uribei*, *P. lehmannii* and *P. cumbalensis* exerted a strong activity (inhibition halo > 40 mm) (Fig. 17 supporting information) while other *Passiflora* samples showed less or no activity at all. The complete results are summarized in Supp. Table 4.

The metabolites that had been detected by ^{1}H-NMR were correlated with the bioactivity (QSI) by applying the orthogonal projection to latent structures (OPLS-DA), using the coded QSI activity (20 mm-inhibition zone was coded as 1; > 30 mm of inhibition zone was coded as 3) as the Y-variable. Separation of the active groups is observed in the OPLS-DA score plot (R^2 = 0.425 and Q^2 = 0.302, pareto scaling), with the active groups on the negative side along OPLS1 (Fig. 7A). *Passiflora cumbalensis* clustered as a well-defined active group, while the other species did not show a clear clustering tendency. Three active groups were identified along the OPLS2 axis, one being on the negative side for *P. lehmannii* and *P. uribei,* one on the positive side for *P. cumbalensis* and a third one for the other species spread out in the middle of the plot, suggesting that the active compounds for these three groups were different.

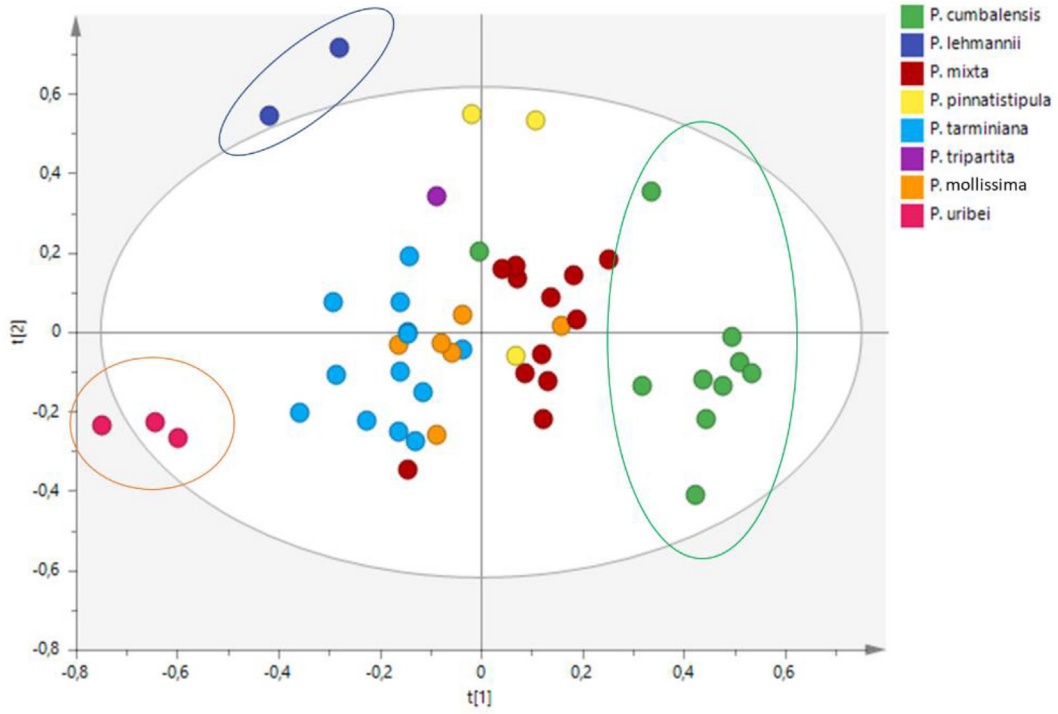

Fig. 6. PCA plot: The score plot of the principal component analysis (PCA) of 8 different species of banana passion fruits species shows a separation into four main groups.

Using two *S-plots*, one excluding *P. lehmanii* samples (Fig. 7B) and the other excluding *P. cumbalensis* samples (Fig. 7C) it was possible to identify the active compounds. The variables important for the projection (VIPs) were selected, and the chemical shifts responsible for the QSI activity were highlighted. These highlighted chemical shifts were found to correspond mostly to the glycosylated flavonoids because the signals could be assigned to aromatic protons such as those of the A and B rings from flavonoids as well as signals for sugar moieties, including those of the anomeric protons close to 5 ppm (Tables 5 and 6, Supporting info).

The quality and robustness of the OPLS-DA model was validated by a permutation test (n = 100). The Q^2 intercept value was −0.504 (below 0.05), showing that the original model was statistically effective (Fig. 18 Supporting info). The model was validated by calculating the area under the receiver operating characteristic (ROC) curve. The value of the area under the curve (AUC) was 0.9565 providing added confidence to the model (Fig. 18B supporting info).

Pure compounds **1** and **2** were tested for their QS inhibition against *C. violaceum* at five concentrations in the range of 50 μM–400 μM in a 96 well-plate. The QS inhibition of compound **1** and compound **2** was detected at concentrations of 100 μg/mL (0.13 mM) and 300 μg/mL (0.47 mM) respectively. In order to establish whether the observed inhibition was due solely to QS inhibition and not to growth inhibition, samples were submitted to a growth inhibition test (Fig. 19 supporting information). Results of the assays showed not only the absence of growth inhibition but an increase in bacterial cell densities, indicating that the flavonoids likely inhibited cell communication.

A second model, *Burkholderia glumae*, a well-known phytopathogen that causes rice grain rot and wilt in various field crops was also used to evaluate QSI (Compant et al., 2008). In *B. glumae*, the production of toxoflavin (a bright yellow pigment) is known to be one of the major virulence factors (Jeong et al., 2003; J. Kim et al., 2004). The biosynthesis of toxoflavin is controlled by ToxR, a LysR-type transcriptional regulator and this toxin also activates the expression of the *tox* operons (J. Kim et al., 2004). For this reason, the search for compounds that are able to inhibit toxoflavin production is an important target for the control of this phytopathogen. Two strains were chosen to determine the toxoflavin inhibitory activity of extracts and pure compounds. *Burkholderia glumae* COK71, is a biosensor strain, that is highly specific for toxoflavin based on β-galactosidase activity on X gal substrate that produces a blue pigment, and the *B. glumae* ATCC 33617 strain as a toxoflavin producer. In this test, the levels of the blue pigment are used to determine toxoflavin inhibitory activity (Choi et al., 2013). Our results indicated that toxoflavin productions was inhibited by concentrations of 6.76 μM and 7.87 μM of compounds **1** and **2,** respectively, while the positive control, 2-*n*-propyl-9-hydroxy-4H-pyrid [1,2-a] pyrimidin-4-one was active at 80 μM, showing the potential of these flavonoids to control toxin production by the phytopathogen, *B. glumae* (Fig. 20, supporting information).

The presence of flavonoids in plant extracts has been previously related to their QS inhibition activity. Phytochemical screening of *Centella asiatica* has revealed that flavonoids can disrupt AHL-mediated QS-controlled systems in *C. violaceum* and *P. aeruginosa* while major constituents such as the triterpene, asiatic acid, did not show an anti-QS activity (Vasavi et al., 2016). Concentrations of 100 μg/mL of quercetin and kaempferol have been reported to exhibit anti-QS activity against *C. violaceum* and *P. aeruginosa* PAO1. The anti-QS activity of *Psidium guajava* leaf extract has been determined with a biosensor bioassay using *Chromobacterium violaceum* CV026, and quercetin and quercetin 3-*O*-arabinoside were identified as the QQ compounds in the extract, against *C. violaceum* 12,472, at concentrations of 50 and 100 μg/mL, respectively (Vasavi et al., 2014). Similarly, Paczkowski et al. studied the QS inhibition mechanism of flavonoids, establishing that they are inhibitors of the QS transcriptional regulator LasR and that they specifically inhibit quorum sensing via antagonism with the transcriptional regulator LasR/RhlR. Further structure-activity relationship analyses suggest that the presence of two hydroxyl moieties in the flavone A-ring backbone are essential for potent inhibition of LasR/RhlR. Biochemical analyses also revealed that flavonoids function non-competitively to prevent LasR/RhlR DNA-binding. The administration of the flavonoids to *P. aeruginosa* was found to alter transcription of the quorum-sensing controlled target promoters and suppress virulence factor production,

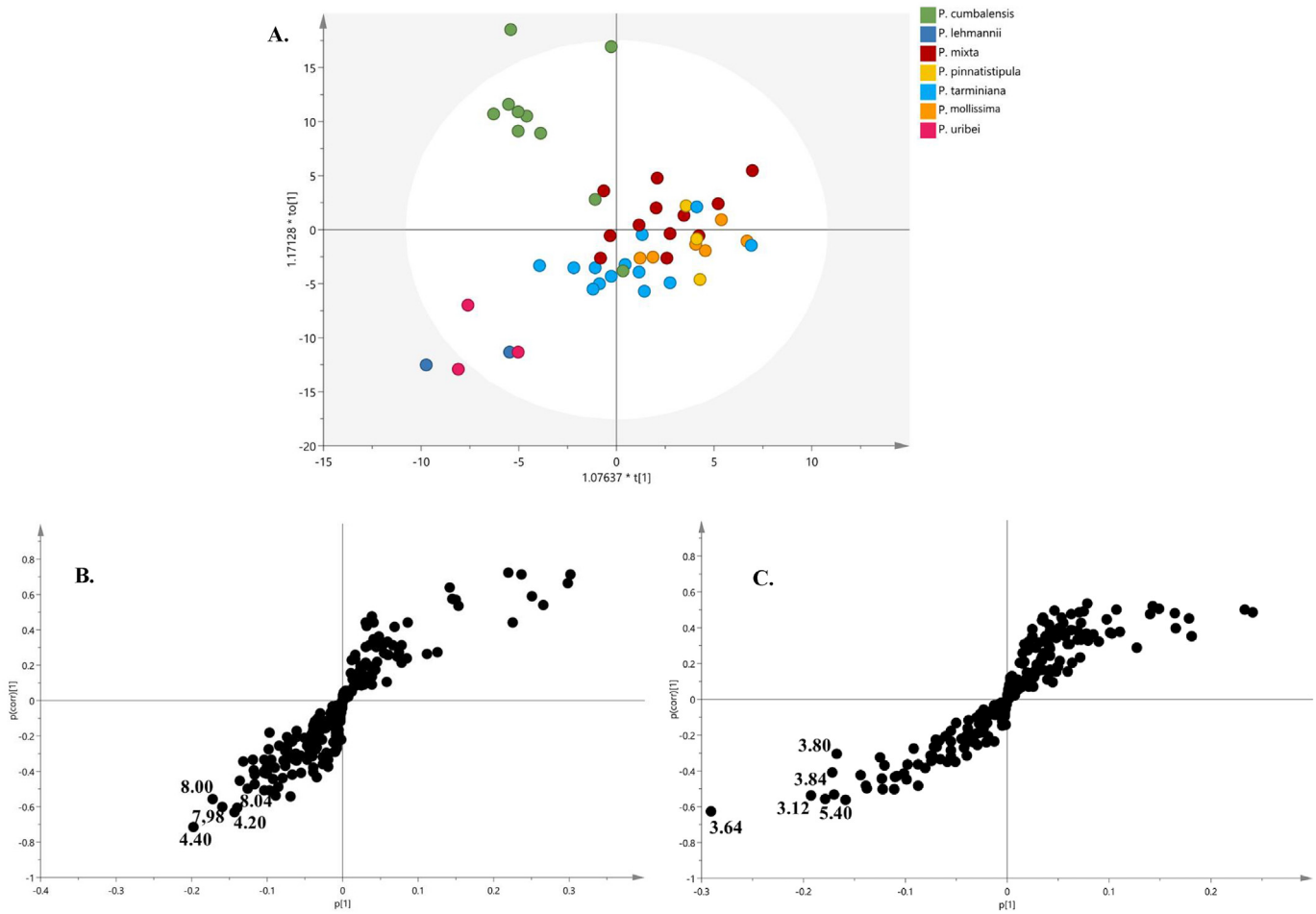

Fig. 7. Supervised multivariate analysis of NMR data. A) OPLS *score plot* using Y-variable of QSI activity. The plot showed the separation of active and non-active samples along OPLS1 (left side active samples). B) The corresponding *S-plot* significant values for QSI activity without *P. lehmanii* samples. C) The corresponding *S-plot* significant values for QSI activity without *P. cumbalensis* samples.

confirming their potential as antimicrobials which do not function by traditional bactericidal or bacteriostatic mechanisms (Paczkowski et al., 2017).

3. Conclusion

Different species of Pa*ssiflora* subgenus were compared using a metabolomics approach This subgenus includes a group of plants with different biological activities, among which anxiolytic activity is the most prominent. The chemical fingerprinting of seven of the species of this subgenus allowed the identification 59 compounds including amino acids, organic acids, flavonoids and other phenolic compounds. Two previously undescribed flavonoids were identified in *P. uribei* and *P. lehemanii* extracts. Multivariate Data Analysis (PCA and OPLS) of the data of the analyzed species showed *P. cumbalensis* and *P. uribei* to be highly distinguishable species among the other studied species (*P. mollisima, P. tarminiana, P. mixta,* and *P. tripartita*). The metabolites that proved to be responsible for these differences were the chrysin glycosides identified in *P. cumbalensis* and acetyl hexoside vitexin present in *P. uribei.* In addition, the flavonoid content was correlated to the *quorum sensing* inhibitory activity using two types of biosensors, *C. violaceum* and *B. glumae*. This research contributed to the establishment of metabolic fingerprints for these *Passiflora* species and their correlation with a biological activity, revealing new potential uses for species of this subgenus. Further chemical studies of the species are required to determine the influence of geographical and environmental conditions on their metabolic profiles.

4. Materials and methods

4.1. General

Ultrasonicator (Branson 5510 E-MT, Branson Ultrasonics, Danbury, CT, USA), microcentrifuge (Thermo Scientific, Heraeus Pico 17), freeze-dryer (Edwards Ltd., Crawley, UK). The 600 MHz (^{1}H frequency) NMR spectra were recorded on a Bruker spectrometer (DMX-600 spectrometer, Bruker AXS Advanced X-ray Solutions GmbH, Karlsruhe, Germany) operating at a ^{1}H NMR frequency of 600.13 MHz and equipped with a TCI cryoprobe and Z-gradient system. The 400 MHz (^{1}H frequency) NMR spectra were recorded on a Bruker spectrometer (Avance 400) operating at a ^{1}H NMR frequency of 400.13 MHz. The UHPLC-TOF-MS analyses were performed on an Ultimate 3000 UHPLC system (Thermoscientific, Pittsburgh, PA, USA) coupled to a micro-ToF-2Q mass spectrometer from Bruker Daltonics (Bremen, Germany) with an ESI interphase. Silica gel pore size 60 Å, 70–230 mesh) for open column chromatography was purchased from Sigma-Aldrich (St. Louis, MO, USA). Sephadex LH-20 (GE Healthcare Life Sciences, Eindhoven, The Netherlands) was used for size-exclusion chromatography.

4.2. Plant material

Plants used for this study belonging to the genus *Passiflora*, family Passifloracea were collected as indicated in Table 1 (more details are provided in Supp. Table 4) and botanically identified according to Escobar, Ocampo and Primot (Primot et al., 2005, Ocampo Pérez and

Coppens d'Eeckenbrugge, 2017). All studied species (banana passion fruits) belong to *Tacsonia* subgenus of *Passiflora* genus. A low-level classification based on phenetic features (Ocampo Pérez and Coppens d'Eeckenbrugge, 2017). A granadilla species, *P. lehmannii* (subgenus *Passiflora,* supersection *Stipulata* Feuillet & MacDougal) was also collected for comparative purposes. The Central region refers to the Cundinamarca department and the Southern region to Nariño and Huila departments, and altitude and GPS data can be consulted in supplementary information.

All samples consisted of six to ten healthy leaves cut with sterile scissors from mature wild type flowering and fruit-bearing plants. The leaves were frozen immediately with liquid nitrogen and stored at −80 °C. After grinding in liquid nitrogen, samples were freeze-dried for 48 h, and then extracted with CH_3OH-d_4 in buffer (90 mM KH_2PO_4 in D_2O) ^{1}H NMR analysis.

4.3. Chemicals and reagents

Methanol-d_4, deuterium oxide, and TSP-d_4 were purchased from Sigma-Aldrich GmbH (Steinheim, Germany). Methanol (AR and Lichrosolv, MerckMillipore), acetonitrile (LCMS grade, Fisher Scientific), formic acid (LCMS grade, Optima, Fisher Scientific), dichloromethane (AR grade) and butanol (AR grade) were purchased from Sigma Aldrich (St. Louis, MO, USA). Reference standards isoorientin (, > 98%), orientin (> 97%), rutin trihydrate (> 95%), isovitexin (> 95%), vitexin (, > 95%), and luteolin-7-O-glucoside (, > 98%) were purchased from Sigma. The identity of the reference compounds was verified by ^{1}H NMR and LC-MS.

4.4. NMR analysis

Samples were prepared for metabolomic plant analysis as described by Kim et al., 2010) with slight modifications as described in the supporting information.

4.5. LC-MS analysis

For LCMS analysis, 500 mg of dried ground *Passiflora* leaves were mixed with 5 mL of water: methanol (1:1) and sonicated at 25 °C for 15 min. The extract was filtered and taken to dryness under reduced pressure. The resulting residue was resuspended in water (5 mL) and extracted subsequently with dichloromethane and butanol. The butanolic fraction was taken to dryness and freeze-dried. Solutions of 1 mg/mL of the butanolic fraction in water were filtered through 0.20 μm disposable membrane filters (Minisart® RC15 Syringe, 0.2 μm). This procedure has been reported to provide flavonoid and saponin-rich butanolic fractions (Zucolotto et al., 2012).

UHPLC-TOF-MS Analysis. Mass spectroscopical analysis was performed on an Ultimate 3000 UHPLC coupled to a Bruker Daltonics microTOF-QII with an ESIsource. In separate runs, detection was done in both positive and negative ion modes. The *m/z* range was 100–1300 Da and the ESI conditions were as follows: nebulizer gas, nitrogen, 2.0 bar; nitrogen drying gas 11 L/min, 200 °C; capillary, 3800 V; end plate offset, −500 V; funnel 1 RF, 220 Vpp; funnel 2 RF, 140 Vpp; collision gas, nitrogen; collision energy, 8 eV; collision RF 300 Vpp; transfer time, 70 μs; prepulse storage, 5 μs; spectra rate, 2 Hz. The mass spectrometer was programmed to acquire MS/MS in a data-dependent manner, consisting in five MS/MS scans following each precursor MS1 scan. Similar conditions were used for positive ionization but the capillary voltage was 4000 V. Calibration was performed using a 10 mM sodium formate solution from Sigma–Aldrich (Steinheim, Germany). Samples (3 μL) were separated on a Kinetex C18, 150 × 2.0 mm column, packed with 2.7 μm particles (Phenomenex, USA) at a flow rate of 0.35 mL/min using a gradient of 0.5% aqueous formic acid (solvent A) and acetonitrile/0.5% formic acid (solvent B), from 17 to 35% A (0–20 min).

4.6. Isolation of flavonoids

Major flavonoids of *P. cumbalensis, P. lehmannii* and *P. uribei* detected in the butanolic fractions were isolated using column chromatography, and each fraction was analyzed by LC-MS and NMR; the assigned peaks were used in the analysis of NMR raw extracts spectra (section 4.3). (For details of isolation please consult supporting information).

4.7. Biological assay

4.7.1. Disruption of Chromobacterium violaceum violacein production

Extracts of *Passiflora* species were obtained by ultrasonication of 20 mg of freeze-dried powdered leaves with 1.0 mL of methanol:water (1:1). Mixtures were then centrifuged for 20 min 6000 rpm, and the supernatants were tested for the presence of QQ compounds using a modified well-diffusion plate assay (Balouiri et al., 2016; McLean et al., 2004). Briefly, a suspension of *Chromobacterium violaceum* ATCC31532 strain, previously grown in LB liquid medium, was inoculated into 30 mL of LB agar medium in order to obtain an OD_{600nm} 0.5 suspension. The medium was then poured into 9 cm agar plates and four wells (6 mm in diameter) were cut from the agar once it solidified. A 300 μL sample of each methanol-water extract was loaded into each well and the plates were incubated for 24 h at 30 °C. An aliquot of 300 μL of MeOH:H_2O (1:1) was used as a negative control and 200 μg of 4-hydroxybenzaldehyde (PHB) or kojic acid were used as positive controls. The presence of quorum quenching compounds was detected by the lack of violacein production around the wells without growth inhibition of the biosensor (*C. violaceum*).

4.7.2. Burkholderia glumae assays by cross-streaking

LB agar plates were supplemented with X-gal (40 mg/mL) and the extract (0.05 mg/mL) or pure compound (5 μg/mL, 6.76 μM and 7.87 μM for **1** and **2**, respectively) to be assayed. An 0.066 mg/mL (80 μM) solution of the quorum quencher 2-*n*-propyl-9-hydroxy-4H-pyrid [1,2-a] pyrimidin-4-one was used as a positive control. This compound was synthetized according the patent N°US 8,507,674 B2 Aug. 13, 2013 (Suga and Igarashi, 2010). As a negative control, *Burkholderia glumae* ATCC33617 and *B. glumae* COK7 were T-streaked onto the same plate with a 5 mm distance between the tip of both streaks. These strains were previously grown in LB liquid medium, and the suspensions were adjusted to OD_{600nm} 0.5. The presence of toxoflavin induces *β*-galactosidase production in strain COK71, developing a blue color. No phenotypic changes were observed in the absence of toxoflavin (Choi et al., 2013). It was thus possible to establish whether the toxoflavin quorum sensing circuit had been quenched by the compounds or extracts The biosensor strain *B. glumae* COK 71 was kindly donated by Professor Jimwoo Kim of the University of Korea (Choi *eta*, 2013).

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2020.112272.

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