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Mechanistic evidence of *Passiflora edulis* (Passifloraceae) anxiolytic activity in relation to its metabolite fingerprint as revealed *via* LC-MS and chemometrics†

Asmaa Otify, Camilia George, Aly Elsayed and Mohamed A. Farag*

Passiflora edulis Sims *F. flavicarpa* along with several other plants belonging to the genus *Passiflora* have been reported as sedatives and for treatment or prevention of central disorders. This study evaluated the anxiolytic effect of *P. edulis* ethanol extract and its fractions (*viz.* chloroform, ethyl acetate and butanol) using the elevated plus-maze model of anxiety and assessment of γ -aminobutyric acid levels. The results revealed that butanol and chloroform extracts exhibit the strongest effect followed by ethyl acetate suggesting that a combination of different classes of metabolites is likely to mediate for *P. edulis* anxiolytic effect in these fractions. To further pinpoint bioactive agents in fractions, ultra-performance liquid chromatography (UPLC) coupled to high resolution qTOF-MS was used for secondary metabolite profiling. A total of 65 metabolites were characterized including *O*-flavonoids, *C*-flavonoids, cyanogenic glycosides and fatty acids. Harman type alkaloids found in *P. incarnata* were not detected in *P. edulis* ethanol extract or any of its fractions suggesting that they do not mediate for its CNS modulating effects. Multivariate data analysis (PCA) was further applied to identify metabolite markers for fractions and revealed that enrichment of *C*-glycoside type flavonoids in chloroform/ethyl acetate fractions *versus* the exclusive presence of cyanogenic glycosides in its butanol fraction.

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Introduction

The genus *Passiflora* contains *ca.* 400 species of flowering plants and is the largest in the family of Passifloraceae. The medical utility of very few *Passiflora* species has been scientifically elucidated. Passion extracts have been found to exhibit a myriad of pharmacological activities including anxiolytic, spasmolytic, hypnotic, sedative, narcotic and anodyne activities, and with their aerial parts (stem and leaf) been used traditionally to enhance the effects of mind-altering drugs.

The neuropharmacological effects of *Passiflora* spp., *i.e.* *Passiflora incarnata*,¹ *P. caerulea*,² and *P. edulis*,³ are thought to be mostly mediated *via* their flavonoid content. Flavonoids are absorbed in the digestive tract, and then transported by the circulatory system to the brain, where they must pass the blood–brain barrier before exerting a CNS effect. Some natural flavones have been shown to possess selective affinity for the benzodiazepine binding site on the GABA_A receptors (GABA_AR) in the brain. GABA_AR is an ionotropic receptor and a ligand-

gated ion channel which binds to its endogenous ligand γ -aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system.⁴ The first flavone described as a specific ligand for the benzodiazepine binding site was chrysin (5,7-dihydroxyflavone)⁵ isolated from *P. caerulea* and was found to be a selective and competitive inhibitor of [³H] flunitrazepam binding to the benzodiazepine binding site. Luteolin is another flavonoid aglycon found in a wide variety of *Passiflora* spp. It has been reported to displace [³H] flunitrazepam from the benzodiazepine binding site *in vitro*, with low affinity, and to exhibit anxiolytic-like effects when administered orally in mice. In addition, a commercial extract of *P. incarnata* enriched with *C*-glycosyl flavones demonstrated, *in vivo*, GABA mediated anxiolytic activity.⁶ Based on structure–activity relationship studies, incorporation of electronegative groups into the C-6 and C-3' of the flavone backbone was found to enhance its binding affinities for the benzodiazepine binding site.^{7,8}

P. edulis is economically the most important species within the genus *Passiflora* and is an edible variety most commonly known for its tasty fruit. There have been several reports on the neuropharmacological activities of *P. edulis*, albeit, the experimental results from different research groups have brought some controversy on the *P. edulis* potential anxiolytic

Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt. E-mail: mfarag73@yahoo.com, mohamed.farag@pharma.cu.edu.eg

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effect. The work by Petry *et al.*⁹ revealed that *P. edulis* extract was active in the rodent model of anxiety, as well as those by Coleta *et al.*¹⁰ and in opposition to Dhawan's¹¹ findings that *P. edulis* was devoid of any anxiolytic activity. These results suggest the need for more thorough investigations on the *P. edulis* effect on the nervous system and or chemical profiling of its secondary product metabolome for proof of action.

The present study aims at evaluating anxiolytic and sedative effects of *P. edulis* ethanol extract (EE) as well as its sub-fractions, *viz.* chloroform (CF), ethyl acetate (EAE), and *n*-butanol (BF) so as to ascertain if this species could be used as a resource material of passion flower extracts or an alternative to *P. incarnata* official drug. The behavioural effects of *P. edulis* aerial part crude ethanol extract and its sub-fractions were assayed using the elevated plus-maze model (EPM) in mice as one of the most popular behavioural tests for assessing anxiolytic drugs. γ -Aminobutyric acid (GABA) levels were also measured in the treated animal brain *versus* control animals to determine whether the GABA-ergic system mediates the *P. edulis* anxiolytic effect.

Furthermore, *P. edulis* crude ethanol extract and sub-fractions used for biological testing were subjected to comprehensive metabolite profiling using UPLC-high resolution qTOF-MS to reveal their chemical composition. Different metabolites including several *C*-flavonoid glycosides were identified in *P. edulis* crude extract and sub-fractions in addition to cyanogenic glycosides and fatty acids. To define both metabolite similarities and differences among fractions and to assist in pinpointing bioactive agents that could correlate with these effects, multivariate data analysis, principal component analysis (PCA) was adopted. Such a metabolomic approach could identify potential active anxiolytic agents in the different examined *P. edulis* extracts.

Materials and methods

Plant materials and chemicals

The aerial parts of *P. edulis* were collected in September 2012, from El-Orman Botanical Garden, Giza, Egypt and authenticated by Mrs Teresa Labib, Head of the Taxonomists at El-Orman Botanical Garden. A voucher specimen (26-12-2012) is deposited in the Herbarium of the Department of Pharmacognosy, College of Pharmacy, Cairo University, Egypt. Solvents used for plant extraction: ethanol, petroleum ether, ethyl acetate, and *n*-butanol were all of analytical grade and purchased from El-Gomhouria Co. (Cairo, Egypt). For the pharmacological assays, GABA was purchased from Sigma-Aldrich (St. Louis, MO, USA) and diazepam from Memphis Pharmaceutical & Chemical Industry (Cairo, Egypt).

Animals

Male Swiss albino mice, weighing 20–35 g were bred at the Animal House of the College of Pharmacy, Cairo University, with free access to food and water in a temperature-controlled room (25 ± 2 °C) for at least 1 week prior to the behavioural

experiments. This study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of Faculty of Pharmacy Cairo University, Cairo, Egypt following the 18th WMA General Assembly, Helsinki, June 1964 and updated by the 59th WMA General Assembly, Seoul, October 2008. Each animal was used just once. The animals were assigned randomly to different treatment groups ($n = 5$).

Extraction and fractionation procedures

1200 g of dried *P. edulis* aerial parts (leaf and stem) was extracted with ethanol–H₂O (70 : 30) at room temp. The solvent was evaporated under vacuum and the residue of the ethanol extract (EE, 70 g) was partitioned between petroleum ether–H₂O, followed by chloroform–H₂O, ethyl acetate–H₂O, and *n*-butanol–H₂O, in turn, to give a petroleum ether fraction (40 g), a chloroform fraction (1.3 g), an ethyl acetate fraction (2 g) and an *n*-butanol fraction (20 g).

Drugs and treatment

EE, CF, EAE and BF were uniformly suspended in distilled water by ultra-sonication with the addition of 1% Tween 80. Diazepam *i.v.* solution (DZ) was diluted with distilled water and used at a dose of 2 mg kg⁻¹, the reference drug for anxiolytic and sedative tests. The trial animals received single administration of a uniform suspension of the different test extracts and fractions. The negative control group received the vehicle (distilled water containing Tween 80) and the positive control group received DZ. All treatments were orally administered (*p.o.*) at a volume of 10 ml kg⁻¹. In the EPM tests, EE was given to mice at two doses, *viz.* 200 and 400 mg kg⁻¹, whereas CF, EAE, and BF were administered to mice at a single dose of 200 mg kg⁻¹.

Pharmacological assays

Elevated plus-maze (EPM). The model is based on the natural aversion of rodents for open spaces and uses conflict between exploration and aversion to elevated open spaces and such a model has been well validated for assessing both rats and mice.¹² The EPM is in the form of a 'plus' with two open elevated arms facing opposite to each other and separated by a central square and two arms of the same dimensions, but enclosed by walls. The maze is raised off the ground so that the open arms combine elements of unfamiliarity, openness and elevation (ESI Fig. 1S†).

The apparatus is composed of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 cm) with a 40 cm high wall arranged so that the arms of the same type are opposite to each other with a central square of 10 cm to form a plus sign. The apparatus is elevated to a height of 50 cm above the floor level by a single central support. To facilitate adaptation to new surroundings, animals are transported to the laboratory at least 1 h prior to testing. At the start of the experiment, each mouse was placed individually at the centre of the maze facing an open arm and allowed for free exploration for a period of 5 min. The conventional spatial-temporal measures recorded

were the numbers of all entries (all four paws on open or enclosed arms), and the percentage of open arm entries (OE%, $100 \times \text{open}/\text{total entries}$) and of the time spent in open arms (OT%, $100 \times \text{open}/(\text{open} + \text{enclosed})$). After each test, the maze is carefully cleaned up with 10% ethanol.¹³

Determination of the brain neurotransmitter (GABA) level.

To assess the possible involvement of the GABA-ergic system, brains of all animal groups, *viz.* negative control, positive drug control (DZ) and treatment groups (EE, CF, EAE and BF) were surgically isolated and evaluated for their GABA content. Each brain tissue was weighed and homogenized in 75% aqueous HPLC grade methanol (10% w/v) (Arafa *et al.*, 2010).¹⁴ The homogenate was spun at 4000 rpm for 10 min and the supernatant was dried using vacuum adjusted at 70 millitorr at room temperature and used for GABA determination. GABA was detected *via* High Performance Liquid Chromatography (HPLC) using the precolumn PITS derivatization technique according to the method of Henrikson and Meredith (1984).¹⁵ Standard GABA was prepared as previously mentioned in brain sample preparation. Quantification of GABA in the HPLC chromatogram of samples was compared to that of the standard and determined as μg content per gram brain tissue ($\mu\text{g g}^{-1}$).

Statistical analysis. Data were analysed with PSPPP, 0.8.2 for Windows (SPSS Inc., Chicago, IL). Data are expressed as mean \pm S.E.M. of the groups. Differences between treatments were compared by one way ANOVA, followed by the Least Significant Difference test (LSD test) for approximately normally distributed variables or the non-parametric Mann–Whitney test. Differences between treated and control groups were considered statistically significant when $P \leq 0.05$ for one way ANOVA and $P \leq 0.1$ for the Mann–Whitney test (due to small sample size).

High-resolution UPLC-PDA-qTOF-MS analysis

Chromatographic separations were performed using an Acquity UPLC system (Waters) equipped with an HSS T3 column (100×1.0 mm, particle size 1.8 μm ; Waters) by applying two elution binary gradients at a flow rate of $150 \mu\text{L min}^{-1}$: (1) 0 to 1 min, isocratic 95% A (water/formic acid, 99.9/0.1 [v/v]), and 5% B (acetonitrile/formic acid, 99.9/0.1 [v/v]); 1 to 16 min, linear from 5 to 95% B; 16 to 18 min, isocratic 95% B; 18 to 20 min, isocratic 5% B. The second binary eluent (2) was composed of ammonium acetate 50 mM buffer adjusted to pH 5 (A) and 100% acetonitrile (B) using the same elution gradient as above. The injection volume was 3.1 μL (full loop injection). Eluted compounds were detected from m/z 100 to 1000 using a MicroTOF-Q hybrid quadrupole time-of-flight mass spectrometer (Bruker Daltonics) equipped with an Apollo II electrospray ion source in both negative and positive ion modes using the following instrument settings: nebulizer gas, nitrogen, 1.6 bar; dry gas, nitrogen, 6 l min^{-1} , 190 $^{\circ}\text{C}$; capillary, -5500 V (+4000 V); end plate offset, -500 V; funnel 1 RF, 200 Vpp; funnel 2 RF, 200 Vpp; in-source CID energy, 0 V; hexapole RF, 100 Vpp; quadrupole ion energy, 5 eV; collision gas, argon; collision energy, 10 eV; collision RF 200/400 Vpp (timing 50/50); transfer time, 70 μs ; prepulse storage, 5 μs ; pulser frequency, 10 kHz; spectral rate, 3 Hz. Internal mass

calibration of each analysis was performed by infusion of 20 μL 10 mM lithium formate in isopropanol/water, 1/1 (v/v), at a gradient time of 18 min using a diverter valve. Metabolites were characterized by their UV-vis spectra (220–600 nm), mass spectra, and comparison with our in-house database and the reference literature.

UPLC-MS data processing for multivariate analysis

The relative metabolite quantification of *P. edulis* metabolite profiles derived using UPLC-MS was performed using XCMS data analysis software, which can be downloaded free as an R package from the Metlin Metabolite Database <https://metlin.scripps.edu/download/>.¹⁶ This software approach employs peak alignment, matching and comparison following the exact procedure described by Farag and Wessjohann.¹⁷ Principal component analysis (PCA) was performed on the MS-scaled data using custom script under the R 2.9.2 environment. The PCA was run for obtaining a general overview of the variance of metabolites. The distance to the model (DModX) test was used to verify the presence of outliers and to evaluate whether a submitted sample fell within the model applicability domain.

Results and discussion

Elevated plus-maze test

Diazepam included in the elevated plus-maze test as a positive drug control induced a selective anxiolytic-like effect in mice characterized by a significant increase ($P \leq 0.1$) in the percentage of open arm entries, OE% and OT%, of the EPM, without changing the number of enclosed arm entries, compared to the negative control (ESI Table 1† & Fig. 1) and was in agreement with previous results.¹⁸ Treatment with *P. edulis* ethanol extract (EE) at 200 and 400 mg kg^{-1} significantly increased the percentage of open arm entries, OE% and OT%, of the mice demonstrating that EE of *P. edulis* exhibited an anxiolytic-like effect. Additionally, a marked decrease in the number of enclosed entries in the EE at 400 mg kg^{-1} treatment groups was observed, suggesting that EE reduced mice motor activity, namely, EE showed anxiolytic activity at a lower dose and more of a sedative-like activity at a high dose. The administration of CF, EAE and BF at 200 mg kg^{-1} resulted in a significant increase in the number of open arm entries, OE% and OT%, without changing the number of enclosed arm entries, compared to the negative control, suggesting an anxiolytic effect of *P. edulis* fractions. However, no significant difference could be observed among the treatment groups. BF was found to manifest the highest activity followed by EAE and CF. (ESI Table 1† & Fig. 1)

Determination of brain (GABA) levels

The results obtained after determination of the brain GABA levels (expressed as μg per g tissue) of the tested animals are expressed as mean \pm S.E.M. and summarized in Fig. 2. Following *P. edulis* extract and fraction administration, a significant increase in the concentration of GABA neurotransmitter at

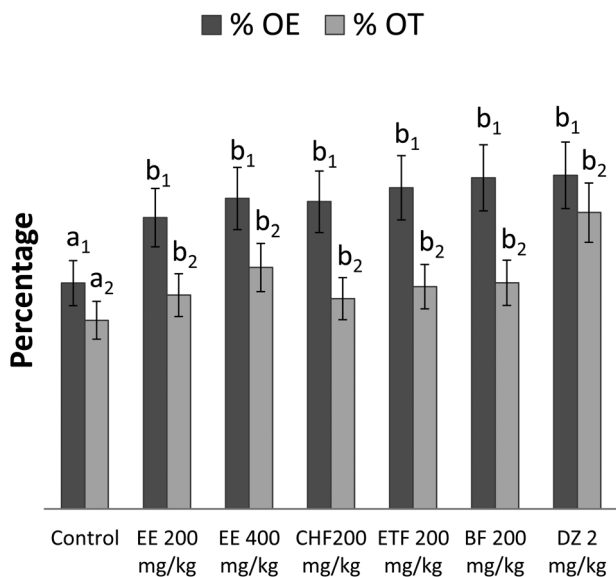


Fig. 1 Effects of the ethanolic extract of *P. edulis* and its fractions on the percentage of open arm entries (OE%), and of the time spent in open arms (OT%) in the elevated plus-maze. (EE) Ethanolic extract, (CHF) chloroform, (EAF) ethyl acetate, (BF) butanol (DZ) diazepam. Results are expressed as mean \pm S.E.M. ($n = 5$). $P \leq 0.1$, compared with control groups (Mann–Whitney test). Different letters indicate significant differences between treatments.

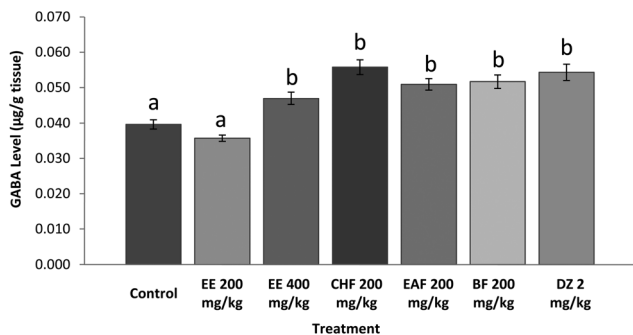


Fig. 2 Effects of the ethanolic extract of *P. edulis* and its fractions on GABA concentration expressed as $\mu\text{g g}^{-1}$ of brain tissue. (EE) Ethanolic extract, (CHF) chloroform, (EAF) ethyl acetate, (BF) butanol (DZ) diazepam. Results are expressed as mean \pm S.E.M. ($n = 5$). $P \leq 0.05$, compared with control groups (one-way ANOVA followed by the Least Significant Difference test (LSD test)). Different letters indicate significant differences between treatments.

$P \leq 0.05$ was revealed compared to the negative control, implying the involvement of the GABA-ergic system in mediating *P. edulis* anxiolytic-like effects, that is, these extracts might function as positive allosteric modulators of GABA and whose actions critically depend on the presence or absence of endogenous neurotransmitters. In detail, CF and BF presented the highest effect at an oral dose of 200 mg kg^{-1} , followed by the EAE fraction. These results were consistent with those revealed from the elevated plus maze model (ESI Table 1† &

Fig. 1). A significant difference exists among each of the examined extracts or fractions, *viz.* EE, CF, EAE, and BF when compared to the negative control. Although no significant difference could be observed among *P. edulis* treatment groups arguing that the anxiolytic activity in *P. edulis* is attributed to the synergistic effect of several active constituents (*i.e.*, flavonoids) rather than a specific single chemical entity. Our results concur with Appel's¹⁹ findings in that the CNS pharmacological effect of *P. incarnata* is mediated *via* modulation of the GABA system including affinity to GABA_A and GABA_B receptors, and the effects on GABA uptake.

Metabolite profiling in *P. edulis* crude ethanol extract and fractions

To assess the metabolite compositions among the different *P. edulis* examined fractions, non-targeted metabolite profiling was conducted for plant crude extracts and their sub-fractions using ultra-performance liquid chromatography coupled to photodiode array and high resolution qTOF-MS operated in both negative and positive ionisation modes.

Following the identification process, 65 chromatographic peaks were detected, of which 33 were tentatively assigned belonging to various metabolite classes. Metabolite assignments were made by comparing retention time, UV-Vis spectra and MS data (accurate mass, isotopic distribution and fragmentation pattern in both negative and positive ionisation modes) and subsequent confirmation by comparison with the literature data. The chromatographic and spectroscopic data are summarized in Tables 1 & 2.

The identified metabolites belonged to various classes, including *O/C* flavonoids, nitriles and fatty acids, with *C*-glycosylated flavones amounting for the most abundant class of secondary metabolites in *P. edulis* and as many as 15 peaks were proposed to represent flavonoid conjugates. Compound abundance in this metabolite group is also visible from the total ion current chromatogram that is rich in the elution region (t_R 170–350 s, Fig. 3A & B).

C/O-Flavonoids

Photodiode array detection (200–600 nm) provided an overview of the main flavonoid constituents in *P. edulis* with flavone glycosidic conjugates representing the major sub-class and found mainly in BF, with a UV maximum absorbance near 270 nm and a second max. between 335 and 350 nm. MS/MS was performed to assist in structural elucidation, where the nature of sugars in *O*-glycosides could be revealed from elimination of the sugar residue, *i.e.*, 162 amu (hexose; glucose or galactose), 146 amu (deoxyhexose), 132 amu (pentose), or 130 (dideoxyhexose) from molecular ions.²⁰ The readily cleaved sugar moieties from aglycon infers *O*-type glycosides, nevertheless in *P. edulis* most flavonoid peaks showed intense molecular ion peaks with $[\text{M}-90\text{-H}]^-$ and $[\text{M}-120\text{-H}]^-$ fragments in the MSⁿ spectrum indicative of sugar cleavage of a *C*-glycoside and found in most identified flavone peaks. The fragmentation pathways most characteristic of *C*-glycosyl flavonoids include dehydration (–18 amu) and cross-ring cleavages

Table 1 Metabolites identified in *P. edulis* ethanol extract using UPLC-PDA-MS in negative ionization mode

Peak no.	M ⁻	R _t (s)	UV	Molecular formula	Error	MS/MS ⁻	Identification
1	456.1499	162	212, 269	C ₂₀ H ₂₆ NO ₁₁ ⁻	1.6	269, 293, 325, 285, 431	Mandelonitrile- <i>O</i> -di-glucoside (amygdalin)
2	502.1570	178	212, 269, 338	C ₂₁ H ₂₈ NO ₁₃ ⁻	0.2	179, 315, 325, 341, 285, 477	2-(4'-Methoxy-3'-hydroxy)-mandelonitrile- <i>O</i> -di-glucoside
3	609.1558	180	214, 269, 346	C ₂₇ H ₂₉ O ₁₆ ⁻	0.8	369, 399, 447, 489, 519, 591	Luteolin-6,8- <i>C</i> -di-glucoside (Lucenin-2)
4	461.1668	190	214, 270, 337	C ₂₀ H ₂₉ O ₁₂ ⁻	0.8	294, 340, 403, 415, 435	Tyrosol- <i>O</i> -di-hexoside
5	593.1651	191	214, 270, 335	C ₂₇ H ₂₉ O ₁₅ ⁻	1.2	353, 383, 473, 503, 575	Apigenin-6,8- <i>C</i> -di-glucoside (vicenin-2)
6	440.1550	193	214, 269, 234	C ₂₀ H ₂₆ NO ₁₀ ⁻	2.0	269, 294, 415, 435	Mandelonitrile- <i>O</i> -rutoside
7	486.1604	199	213, 270, 337	C ₂₁ H ₂₈ NO ₁₂ ⁻	0.7	294, 315, 340, 461	2-(4'-Methoxy-3'-hydroxy)-mandelonitrile- <i>O</i> -rutoside
8	447.1030	202	212, 269, 349	C ₂₁ H ₁₉ O ₁₁ ⁻	0.2	327, 357, 369, 285, 545	Luteolin-6- <i>C</i> -glucoside (isoorientin)
9	563.1512	203	211, 270, 349	C ₂₆ H ₂₇ O ₁₄ ⁻	1.3	443, 473, 383, 503, 487, 547	Apigenin-6- <i>C</i> -glucoside-8- <i>C</i> -arabinoside (schaftoside)
10	447.1030	205	212, 269, 348	C ₂₁ H ₁₉ O ₁₁ ⁻	0.8	327, 357, 285, 545	Luteolin-8- <i>C</i> -glucoside (orientin)
11	577.1444	211	214, 270, 343	C ₂₇ H ₂₉ O ₁₄ ⁻	3.4	457, 487, 559, 311, 473	Chrysin-6,8- <i>C</i> -di-hexoside
12	431.0971	221	214, 270, 333	C ₂₁ H ₁₉ O ₁₀ ⁻	1.0	311, 341, 353, 383, 413	Apigenin-6- <i>C</i> -glucoside (isovitexin)
13	563.1391	230	215, 270, 338	C ₂₆ H ₂₇ O ₁₄ ⁻	3.1	459, 473, 545, 369, 443, 503	Luteolin-6- <i>C</i> -deoxyhexoside-8- <i>C</i> -pentoside
14	561.1717	234	211, 270, 321	C ₂₇ H ₂₉ O ₁₃ ⁻	0.8	269, 311, 415, 547	Apigenin- <i>C</i> -pentoside- <i>O</i> -deoxyhexoside-methyl ether
15	577.1444	235	215	C ₂₇ H ₂₉ O ₁₄ ⁻	2.6	311, 269, 415, 457, 559, 369	Apigenin- <i>C</i> -deoxyhexoside- <i>O</i> -hexoside
16	431.1018	240	212, 270, 347	C ₂₁ H ₁₉ O ₁₀ ⁻	2.7	285, 327, 357, 413, 311, 341	Luteolin-6- <i>C</i> -quinovoside/fucoside
17	415.1039	244	214, 270, 315	C ₂₁ H ₁₉ O ₉ ⁻	2.8	295, 325, 357, 397, 327, 415	Chrysin-6- <i>C</i> -hexoside
18	561.1717	257	215, 270, 325	C ₂₇ H ₂₉ O ₁₃ ⁻	2.4	295, 457, 543, 441	Chrysin- <i>C</i> -hexoside- <i>O</i> -deoxyhexoside
19	431.1128	264	211, 269, 347	C ₂₁ H ₁₉ O ₁₀ ⁻	1.0	285, 327, 357, 311, 413	Luteolin-8- <i>C</i> -deoxyhexoside
20	607.1675	273	212, 269, 341	C ₂₈ H ₃₁ O ₁₅ ⁻	1.1	362, 408, 493	Unknown
21	415.1039	276	217, 269, 330	C ₂₁ H ₁₉ O ₉ ⁻	1.6	269, 294, 311, 341, 397	Apigenin-8- <i>C</i> -deoxyhexoside
22	905.2747	282	217, 271	C ₃₈ H ₆₃ O ₁ ⁻	3.8	545, 743, 811, 859	Unknown
23	383.1335	290	216	C ₁₈ H ₂₃ O ₉ ⁻	3.1	180, 249, 317	Unknown
24	362.1285	296	208, 220	C ₁₈ H ₂₀ NO ₇ ⁻	3.6	225, 256, 294, 408(formic acid adduct)	Mandelonitrile- <i>O</i> -butenylglucoside
25	743.4332	299	217, 270, 346	C ₃₈ H ₆₃ O ₁₄ ⁻	3.9	415, 561, 643	Unknown
26	327.2167	313	209, 274	C ₁₈ H ₃₁ O ₅ ⁻	2.6	113, 215, 257, 269, 301	Trihydroxy-octadecadienoic acid (C _{18:2})
27	889.4931	317	218	C ₄₄ H ₇₅ O ₁₈ ⁻	1.4	395, 467, 765, 809, 843	Unknown
28	741.4039	319	217, 270, 323	C ₃₈ H ₆₁ O ₁₄ ⁻	3.1	327, 399, 697	Unknown
29	329.2320	329	215, 276	C ₁₈ H ₃₃ O ₅ ⁻	1.2	183, 227, 249, 259, 309	Trihydroxy-octadecaenoic acid (C _{18:1})
30	287.2215	342	218	C ₁₆ H ₃₁ O ₄ ⁻	4.6	175, 241, 248, 251, 256	Dihydroxy-hexadecanoic acid (C _{16:0})
31	727.4248	361	218	C ₄₅ H ₅₀ O ₈ ⁻	4.0	249, 317, 475, 533, 667	Unknown
32	253.0497	376	218	C ₁₅ H ₉ O ₄ ⁻	4.3	175, 181, 242, 249	4',7'-Dihydroxyflavone (chrysin)/5,7-dihydroxyneoflavone (serratin)
33	311.1854	380	217	C ₁₇ H ₂₇ O ₅ ⁻	2.7	175, 249	Unknown
34	293.1761	389	218	C ₁₇ H ₂₅ O ₄ ⁻	2.0	175, 236, 243	Unknown
35	487.3400	398	220	C ₃₀ H ₄₇ O ₅ ⁻	5.6	207, 250, 265, 309, 355	Unknown
36	357.2634	402	220	C ₂₀ H ₃₇ O ₅ ⁻	3.3	113, 175, 249, 309	Unknown
37	883.4150	408	220	C ₆₅ H ₅₅ O ₃ ⁻	5.0	113, 175, 249, 309	Unknown
38	721.3617	429	220	C ₃₄ H ₅₇ O ₁₆ ⁻	0.2	649, 675	Unknown
39	313.0775	449	217	C ₁₀ H ₁₇ O ₁₁ ⁻	0.5	113, 175, 249	Unknown
40	293.2120	454	220	C ₁₈ H ₂₉ O ₃ ⁻	0.7	275	Hydroxy-octadecatrienoic acid (C _{18:3})
41	577.1444	461	220	C ₃₀ H ₄₁ O ₁₁ ⁻	4.9	361, 505, 551, 564	Unknown
42	265.1483	465	220	C ₁₂ H ₂₅ O ₄ S ⁻	2.2	243, 249, 253	Unknown
43	559.3105	468	221	C ₂₈ H ₄₇ O ₁₁ ⁻	2.2	297, 339, 487, 513	Unknown
44	699.3792	473	220	C ₂₈ H ₄₇ O ₁₁ ⁻	6.0	291, 297, 559, 595, 653	Unknown
45	595.2880	475	220	C ₄₁ H ₃₉ O ₄ ⁻	3.6	297, 365, 452, 559	Unknown
46	297.1522	476	220	C ₁₉ H ₂₁ O ₃ ⁻	9.4	147, 175, 249, 265	Unknown
47	540.3291	479	220	C ₃₉ H ₄₂ NO ⁻	3.3	295, 363, 417, 480	Unknown
48	295.2276	483	220	C ₁₈ H ₃₁ O ₃ ⁻	0.3	221, 277	Hydroxy-octadecadienoic acid (C _{18:2})
49	561.3255	498	220	C ₂₈ H ₄₉ O ₁₁ ⁻	5.0	249, 311, 471, 539	Unknown
50	571.2870	499	220	C ₃₉ H ₃₉ O ₄ ⁻	4.4	311, 471, 515, 561	Unknown
51	579.2829	500	220	C ₃₀ H ₄₃ O ₁₁ ⁻	4.1	293, 561	Unknown
52	311.1679	506	220	C ₁₃ H ₂₇ O ₈ ⁻	9.0	293, 297	Unknown
53	555.2819	517	220	C ₂₈ H ₄₃ O ₁₁ ⁻	1.7	395, 419, 433, 507	Unknown
54	311.1679	520	220	C ₁₃ H ₂₇ O ₈ ⁻	9.0	165, 249	Unknown
55	555.2829	530	220	C ₂₈ H ₄₃ O ₁₁ ⁻	1.7	433	Unknown
				C ₃₉ H ₃₇ O ₃ ⁻			
56	353.1998	539	220	C ₁₉ H ₂₉ O ₆ ⁻	8.4	285, 325,	Unknown
57	483.2716	546	220	C ₃₆ H ₃₅ O ⁻	5.6	325, 393	Unknown
58	325.1838	556	220	C ₁₄ H ₂₉ O ₈ ⁻	7.6	293	Unknown
59	271.2275	570	220	C ₁₆ H ₃₁ O ₃ ⁻	0.5	113, 265	Hydroxy-hexadecanoic acid (C _{16:0})
60	277.2172	574	220	C ₁₈ H ₂₉ O ₂ ⁻	0.5	211, 251	Linolenic acid (C _{18:3})
61	279.2331	606	220	C ₁₈ H ₃₁ O ₂ ⁻	2.9	171, 211	Linoleic acid (C _{18:2})

Table 1 (Contd.)

Peak no.	M^-	R_t (s)	UV	Molecular formula	Error	MS/MS $^-$	Identification
62	339.1995	633	220	$C_{22}H_{27}O_3^-$	8.8	181, 323	Unknown
63	255.2311	633	220	$C_{16}H_{31}O_2^-$	0.7	239	Palmitic acid ($C_{16:0}$)
64	281.2484	642	220	$C_{18}H_{33}O_2^-$	0.6	171, 183, 239	Oleic acid ($C_{16:1}$)
65	283.2484	680	220	$C_{18}H_{35}O_2^-$	5.7	113, 165, 183, 223, 239	Stearic acid ($C_{18:0}$)

Table 2 MS n data of mono-*C*- and di-*C*-glycosylflavone derivatives in *P. edulis*

Peak no.	M^-	Identification	(M-18) $^-$	(M-90) $^-$	(M-120) $^-$	(M-60) $^-$	(M-104) $^-$
3	609.1558	Luteolin-6,8- <i>C</i> -di-glucoside (lucenin-2)	+	+	++	—	—
5	593.1651	Apigenin-6,8- <i>C</i> -diglucoside (vicenin-2)	+	+	++	—	—
8	447.1030	Luteolin-6- <i>C</i> -glucoside (isoorientin)	+	+	++	—	—
9	563.1512	Apigenin-6- <i>C</i> -glucoside-8- <i>C</i> -arabinoside (schaftoside)	+	+	++	+	—
10	447.1030	Luteolin-8- <i>C</i> -glucoside (orientin)	—	+	++	—	—
11	577.1444	Chrysin-6,8- <i>C</i> -di-hexoside	+	+	++	—	—
12	431.0971	Apigenin-6- <i>C</i> -glucoside (isovitexin)	+	+	++	—	—
13	563.1391	Luteolin-6- <i>C</i> -rhamnoside-8- <i>C</i> -pentoside	+	+	—	+	++
15	577.1444	Apigenin- <i>C</i> -deoxyhexoside- <i>O</i> -hexoside	+	—	—	—	++ ^a
16	431.1018	Luteolin-6- <i>C</i> -deoxyhexoside	+	—	—	—	+
17	415.1039	Chrysin-6- <i>C</i> -hexoside	+	+	++	—	—
18	561.1717	Chrysin- <i>C</i> -hexoside- <i>O</i> -deoxyhexoside	+ ^b	+ ^c	++ ^d	—	+
19	431.1128	Luteolin-8- <i>C</i> -deoxyhexoside	+	—	+	—	++
21	415.1039	Apigenin-8- <i>C</i> -deoxyhexoside	+	—	—	—	+

++ Base peak. ^a (M-hexose-104) $^-$. ^b (M-deoxyhexose-18) $^-$. ^c (M-deoxyhexose-90) $^-$. ^d (M-deoxyhexose-120) $^-$.

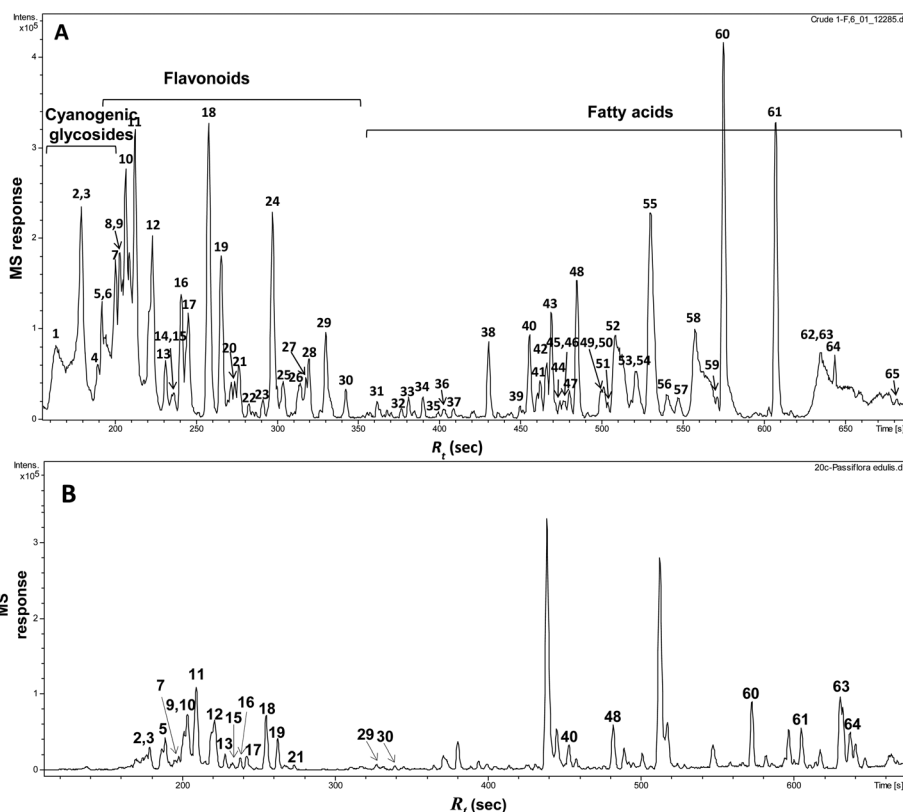


Fig. 3 Representative UPLC-MS traces of *P. edulis* alcohol extract in negative ion mode (A) and positive ion mode (B), the spectrum is characterized by 3 main regions: (50–200 s) with peaks principally due to cyanogenic glycosides, the region (250–350) for flavonoids and the region (350–700) for fatty acids. Peak numbers follow those listed in Table 2 for metabolite identification using UPLC-PDA-MS.

(O,2 and O,3) of the sugar moiety, *i.e.*, (−120 amu) and (−90 amu) for *C*-hexosides and (−90 amu) and (−60 amu) for *C*-pentosides.^{21,22}

In *P. edulis*, mono-*C* and di-*C*-glycosides identified were all flavone derivatives of apigenin and luteolin on the basis of UV-Vis spectra. The ions [Agl + 41/Agl + 71][−] in mono-*C* and [Agl + 83/Agl + 113][−] in di-*C* glycosides typify the aglycon (Agl), that is, [311/341][−] and [353/383][−] for apigenin mono-*C* and di-*C*-glycosides, respectively, [295, 325][−] and [337, 367][−] for chrysin mono-*C* and di-*C*-glycosides, respectively, and ions [327/357][−] and [369/399][−] for luteolin mono-*C* and di-*C*-glycosides respectively.²³ Such ions were identified in apigenin containing peaks (5, 9, 12, 15, and 21), chrysin containing peaks (11, 17 and 18), and luteolin conjugates in peaks 3, 8, 10, 13, 16, 17, & 19, out of which peaks 9, 11, 13, 18, & 19 are reported for the first time in *P. edulis* leaves. The following outline the conditions used to identify each of these flavonoid peaks.

The ESI-MS spectrum of peak 5 exhibited a molecular ion [M − H][−] at *m/z* 593.1651 (C₂₇H₂₉O₁₅)[−] and product ions at *m/z* 575 [M-18-H][−] (−H₂O), 503 [M-90-H][−], 473 [M-120-H][−], 353 [Agl + 83][−], and 383 [Agl + 113][−], a typical fragmentation pattern of flavone di-*C*-hexoside, and was identified as apigenin-6,8-*C*-di-glucoside, also known as vicenin-2.²⁴ Apigenin-6-*C*-monoglucoside was detected in peak 12 from its [M − H][−] at *m/z* 431.0971 (C₂₁H₁₉O₁₀)[−] and fragment ions at *m/z* 413 [M-18-H][−] and *m/z* 341 [M-90-H][−], and a base peak at *m/z* 311 [M-120-H][−], 353 [Agl + 41][−], and 383 [Agl + 71][−], a typical fragmentation pattern of flavone mono-*C*-hexoside (isovitexin), a widespread flavone conjugate in *Passiflora* spp.²⁵

Peak 9 was characterized by an [M − H][−] at *m/z* 563.1512 (C₂₆H₂₇O₁₄)[−] and fragment ions in the MS/MS spectrum appearing at *m/z* 545 [M-18-H][−], *m/z* 473 [M-90-H][−], and 443 [M-120-H][−], a typical fragmentation pattern of flavone-*C*-hexoside, in addition to a fragment at *m/z* 503 [M-60-H][−] indicating the presence of an extra *C*-linked pentose moiety. A base peak at *m/z* 459 [M-120-H] and the high abundance of the fragmentation peaks for the hexosyl unit relative to the pentosyl, suggested that a hexose at position 6 occurs in this compound. Consequently, this *C*-glycoside was identified as apigenin-6-*C*-glucoside-8-*C*-arabinoside, also known as schaftoside.²⁶

Two apigenin isomer conjugates were found in peaks 11 and 15 with [M − H][−] at *m/z* 577.1444 (C₂₇H₂₉O₁₄)[−]. For peak 11, the MS/MS spectrum of deprotonated molecular ions produced ions at *m/z* 559 [M-18-H][−], *m/z* 487 [M-90-H][−], and a base peak at *m/z* 457.1 [M-120-H][−], a fragmentation pattern of flavone-di-*C*-hexoside. This flavone was characterized as chrysin-6,8-di-*C*-hexoside. In contrast, peak 15 exhibited a fragmentation pattern with an intense ion at *m/z* 415 [M-162-H][−] resulting from the loss of the hexose moiety and suggesting an *O*-glycosidic linkage, and a base peak at *m/z* 311 [M-hexose-104-H][−] indicative of a *C*-deoxyhexose moiety. Accordingly peak 15 was identified as apigenin-*C*-deoxyhexoside-*O*-hexoside, previously reported in a *P. edulis* leaf.²⁷

Peak 18 was characterized by a [M − H][−] at *m/z* 561.1717 (C₂₇H₂₉O₁₃)[−] and with product ions at *m/z* 543 [M-18-H][−], 415

[M-deoxyhexose-H][−], *m/z* 325 [M-deoxyhexose-90-H][−], and a base peak at *m/z* 295 [M-deoxyhexose-120-H][−], exhibiting a fragmentation pattern of flavone-*C*,*O*-di-glycoside which was characterized as chrysin-*C*-hexoside-*O*-deoxyhexoside.

Peak 21 with [M − H][−] at *m/z* 415.1039 (C₂₁H₁₉O₉)[−] exhibited a fragmentation pattern indicative of a mono-*C*-glucoside, that is, *m/z* 397 [M-18-H][−], *m/z* 325 [M-90-H][−] and *m/z* 295 [M-120-H][−]. The low abundance of *m/z* 397 [M-18-H][−] suggests an 8-*C* rather than 6-*C*-linked sugar. Consequently peak 21 was identified as apigenin-8-*C*-deoxyhexoside, previously reported in *P. edulis*.²⁷

Peak 3 exhibited an [M − H][−] at *m/z* 609.1558 (C₂₇H₂₉O₁₆)[−] and its MS^{*n*} spectrum showed ions at *m/z* 489 [M-120-H][−], *m/z* 519 [M-90-H][−], and *m/z* 591 [M-18-H][−] corresponding to the fragmentation of a flavone-di-*C*-glycoside, and characterized as luteolin 6,8-di-*C*-glucoside (lucenin-2) previously isolated from the leaves of *P. edulis* *F. flavicarpa*.¹⁸

Other *C*-glycosides identified in *P. edulis* include luteolin-6-*C*-glucoside (isoorientin) peak 8, and luteolin-8-*C*-glucoside (orientin) peak 10, with [M − H][−] of 447.1030 (C₂₁H₁₉O₁₁)[−] and a base peak at *m/z* 327 [M-120-H][−] characteristic of *C*-hexosides. Distinguishing between both isomers was based on the high intensity of the ion *m/z* 429 [M-18-H][−] in peak 8 indicating 6-*C*-hexosyl flavone (isoorientin), due to the formation of an additional hydrogen bond between the 2''-hydroxy group of sugar and the aglycon 5- or 7-hydroxy group which confers additional rigidity.^{22,28} Such ions showed less abundance in luteolin-8-*C*-glucoside (orientin), peak 10. Both compounds are of common occurrence in *Passiflora* spp.²⁵

Peak 13 was characterized by an [M − H][−] at *m/z* 563.1391 (C₂₆H₂₇O₁₄)[−] and product ions at *m/z* 545 [M-18-H] and a base peak at *m/z* 459 [M-104-H][−] indicating a 6-*C*-deoxyhexosyl moiety. Additional ions at *m/z* 503 [M-60-H][−] characteristic of *C*-pentosides identified peak 13 as luteolin-6-*C*-deoxyhexoside-8-*C*-pentoside.

Peaks 16 and 19 MS spectra exhibited a deprotonated [M − H][−] at *m/z* 431.1018 (C₂₁H₁₉O₁₀)[−] and an intense peak at *m/z* 327 [M-104-H][−] indicating a *C*-deoxyhexose moiety. Distinguishing between both isomer peaks was based on mass loss [M-18-H][−] very intense in peak 16 indicating 6-*C*-glycoside, albeit detected at low intensities in peak 19 indicating 8-*C*-glycoside, accordingly peak 16 was annotated as luteolin-6-*C*-quinovoside or luteolin-6-*C*-fucoside, previously reported in *P. edulis* *F. flavicarpa*, whereas peak 19 as luteolin-8-*C*-deoxyhexoside.²⁹

Peak 17 exhibited an [M − H][−] at *m/z* 415.1039 (C₂₁H₁₉O₉)[−] and its MS^{*n*} spectrum showed ions at *m/z* 397 [M-18-H][−], *m/z* 325 [M-90-H][−] and a base peak at 295 [M-120-H][−], a fragmentation pattern typical of flavone-*C*-hexoside. The low abundance of *m/z* 397 [M-18-H][−] suggests 8-*C* rather than a 6-*C*-linkage, thus compound 17 was characterized as chrysin-6-*C*-hexoside.³⁰

It should be noted that identified *C*-flavone conjugates were found most enriched in BF and EAE (ESI Fig. 2S†) both of which showed potent anxiolytic activity especially in BF, as previously described, and accordingly it is likely that the flavone-

C-glycoside enrichment in these fractions contribute to the anxiolytic activity of *P. edulis*. This assumption is in accordance with the previous studies on flavone-*C*-glycosides isolated from other plants. The work by de Oliveira³¹ proved that vitexin, isovitexin and diosmetin-6-*C*-glucoside from *Erythrina falcata*, a known anxiolytic plant resulted in a significant retention of fear memory and could represent potential therapeutic approaches for the treatment of neurocognitive disorders. Standard orientin, isorientin, vitexin and isovitexin were previously assessed for behavioural effects in mice and are reported to exhibit anxiolytic activity in treated animals³² and our results provide further evidence for their roles in modulating CNS effects.

Cyanogenic glycosides

Cyanogenesis is widespread in plants, but relatively few cyanogenic compounds have been isolated and characterized. In *P. edulis* crude ethanol extract (EE), four cyanogenic glycosides were identified in peaks 1, 2, 6 & 7 as evident from the loss of the cyano moiety (−26 amu) in their tandem MS spectra. Peak 1 with a molecular ion of *m/z* 456.1499 (C₂₀H₂₆NO₁₁)[−] and fragment ions at 431 derived from the loss of the cyano group [M-26-H][−], *m/z* 293 corresponds to the loss of glucose [M-162-H][−] from the molecular ion, in addition to the fragment ion *m/z* at 269 [M - (26 + 162)][−] was identified as mandelonitrile-*O*-di-glucoside (2-hydroxy-2-phenyl-acetonitrile-*O*-di-glucoside) previously isolated from *P. edulis*.³³ Likewise, peak 6 at *m/z* 440.1550 (C₂₀H₂₆NO₁₀)[−] was identified as mandelonitrile-*O*-rutinoside exhibiting the same fragmentation pattern with product ions at *m/z* 415 [M-CN-H][−], *m/z* 294 [M-rhamnose-H][−] and 269 [M-(rhamnose + CN)-H][−] known to occur in *P. edulis*.³⁴ It should be noted that cyanogenic glycosides were found most enriched in BF among other fractions, considering its hydrophilic character.

For peaks 2 at *m/z* 502.1570 (C₂₁H₂₉NO₁₃)[−] and 7 at *m/z* 486.1604 (C₂₁H₂₉NO₁₂)[−], both MS spectra showed the fragments characteristic of phenyl acetonitriles, albeit, with a mass difference of 46 amu compared to that in peaks 1 and 6, respectively, indicating extra methoxy (30 amu) and hydroxy (16 amu) groups. Peaks 2 and 7 were tentatively assigned as 2-(4'-methoxy-3'-hydroxy) derivatives of peaks 2 and 6, respectively, and are reported for the first time in the genus *Passiflora*. Whether cyanogenic glycosides can influence the anxiolytic effect of *P. edulis* *C*-flavonoids has yet to be fully examined.

Fatty acids

In the second half of the chromatographic run (350–800 s), particularly in PEF, MS spectra of several unsaturated fatty acids, that is, oleic 64, linoleic 61, and linolenic acid 60 were identified, as evident from high resolution mass at 281.2484, 279.2331 and 277.2172 with predicted molecular formulae of (C₁₈H₃₃O₂)[−], (C₁₈H₃₁O₂)[−], and (C₁₈H₂₉O₂)[−] respectively and with oleic acid being the most abundant fatty acid. The abundance of such a metabolite type in petroleum ether is expected considering its lipophilic nature, extracting mostly non-polar metabolites. MS signals were also assigned for saturated fatty

acids, that is, palmitic 63 and stearic acids 65, as evident from high resolution masses 255.2311 and 283.2484 with predicted molecular formulae of (C₁₆H₃₁O₂)[−] and (C₁₈H₃₅O₂)[−] respectively. Negative ionisation MS also revealed for several unexpected hydroxylated fatty acids, major peaks (26, 30, 29, 40, 48, & 59). In detail, peaks 40 and 48 showed a mass weight of 293.2120 amu and 295.2276 amu, with predicted molecular formulae of (C₁₈H₂₉O₃)[−] and (C₁₈H₃₁O₃)[−] respectively. A mass difference of 2 amu between 40 and 48 is indicative of an extra double bond and they are tentatively identified as hydroxyoctadecatrienoic acid and hydroxyoctadecadienoic acid (oxylipids). Similarly peaks 26 and 29 showed a mass weight of and 329.2320 and 327.2167 amu, with predicted molecular formulae of (C₁₈H₃₃O₅)[−] and (C₁₈H₃₁O₅)[−], respectively, and with a mass difference of 2 amu suggestive of an extra double bond and they are tentatively identified as trihydroxyoctadecadienoic acid and trihydroxyoctadecaenoic acid. Other oxygenated fatty acids were detected in peaks 30 and 59 showing a mass weight of 287.2215 and 271.2275 amu with predicted molecular formulae of (C₁₆H₃₁O₅)[−] and (C₁₆H₃₁O₄)[−], respectively, and a loss of water molecules (18 amu), suggestive of an extra hydroxy group, and tentatively assigned as dihydroxyhexadecanoic and hydroxyhexadecaenoic acid. This is the first report for the presence of oxygenated fatty acids in *P. edulis* and suggests that UPLC-MS represents another platform for fatty acid profiling in *P. edulis*. There is increasing interest in hydroxylated fatty acids, due to their anti-inflammatory, anti-microbial and cytotoxic activities.^{35,36}

Alkaloids

Harmala indole alkaloids are reported to be present in small amounts in *Passiflora* spp.²⁵ Alkaloids show a much higher response in positive versus negative ionisation which highlights the importance of acquiring MS data in both negative and positive ion modes. Nevertheless, no alkaloids were detected in the positive mode in any of the examined fractions suggesting that the anxiolytic activity of *P. edulis* is attributed to constituents other than the harmala alkaloids. It should be noted that standard harmine alkaloid analysed under the same analytical conditions was detected at a level of up to 1 μg ml^{−1}.

To the best of our knowledge, this UPLC-MS fingerprint provides the first comprehensive profile of the secondary metabolite composition in *P. edulis* and helps provide chemical based evidence for its potential anxiolytic effect.

Quantification of flavonoids via UPLC-MS

Luteolin and apigenin conjugates were quantified using calibration curves derived from MS detection of luteolin and apigenin standards, respectively. The standard for each was prepared at concentrations spanning from 0.1, 1, 10 and 100 μg ml^{−1} and assays were carried out from three independent biological replicates. The results revealed the enrichment of *C*-glycosyl flavones in *P. edulis* aerial parts. Details on the quantification of flavonoids are provided in ESI Table 2.† Pharmacopoeias exclude *P. edulis* aerial portion as the

negative ionisation mode. The main principal component (PC) to differentiate between fractions (PC1) accounts for 39% of variance and PC2 for 31%. PC1 plotted against the PC2 score plot (Fig. 4A) shows that four major distinct clusters are formed, corresponding to the four fractions examined. PEF is positioned on the right-hand side of the plot (positive PC1 values), whereas BF and aqueous mother liquor (AQF) samples are located on the far left-hand side (negative PC1 values), and other samples for CF, EAE and EE are spread in between. Discrimination of CF and EAE samples from the EE ones is also possible along PC2. Such sample segregation is clearly dependent on the solvent polarity effect with lipophilic fractions (BF and AQF) joined together *versus* the hydrophilic ones. The separation observed in PCA can be explained in terms of the compounds identified, using the loading plots (Fig. 4B) contributing peaks and their assignments could be made. Two major groups stand out in these plots. The first corresponds to the MS signals for mandelonitrile glycosides in peaks 1, 2, 6, & 7 and chrysin-6,8-*C*-di-hexoside 11 contributing negatively to PC1 and more enriched in BF. The second, from MS signals assigned to the unsaturated fatty acids namely linolenic 60 and linoleic acid 61 having a positive effect on PC1 contributed the most to the discrimination of PEF. Examination of the PC2 loading plot revealed that variables referred to oxygenated fatty acids, namely, trihydroxyoctadecadienoic acid 26 and trihydroxyoctadecaenoic acid 29 contributed the most to the discrimination of CF from EAE, latter being distinctly rich in flavone mono-*C*-glycosides, *i.e.*, isovitexin 12 and orientin 10.

Conclusions

In conclusion, this work represents the first attempt to rationalize using modern metabolite profiling the use of *P. edulis* in the treatment of CNS disorders, well reported within that genus. Using the described UPLC-qTOF-MS/MS fingerprinting method, a total of 33 different metabolites including 12 *C*- and 4 *C,O*-flavone conjugates were annotated in *P. edulis* crude extract and its sub-fractions. Flavone and its 6/8 substituted derivatives have been shown to possess affinity for the GABA_AR and to exhibit anxiolytic or sedative activity in other plants *i.e.* *Scutellaria baicalensis*, *Leptospermum scoparium*, and *Valeriana wallichii*.³⁹ The metabolomic approach described herein using UPLC-PDA-ESI-MS data and multivariate data analysis helps reveal the major secondary metabolites that contribute to the discrimination of fractions. A range of *C*-flavonoid glycosides were documented from the leaves of *P. edulis*., providing a valuable basis for the search for bioactive CNS drugs in that plant group and allowing the specific search for promising species and ecotypes for particular compounds or groups of compounds. An attempt was made to correlate between individual flavonoids (Table 1) and the measured activities to assist in pinpointing active agents likely to mediate for these effects in *P. edulis*. However, no correlation could be found for either assay, suggesting the additive or synergistic effect of several entities that may eventually be more relevant for the

anxiolytic effects of *P. edulis* than just the enrichment of certain *C*-flavonoid entity. Nevertheless, it should be noted that these findings are just a hint and would have to be followed by more detailed studies of *P. edulis* extracts *in vivo* assessing other anxiolytic treatment models, or ideally by studies on single, isolated compounds to be conclusive. Chemical profiling *via* UPLC-MS revealed the enrichment of di-*C*-glycosides *versus* mono-*C*-glycosides in BF and EAE that are likely to mediate the observed anxiolytic effect in these matrices. Whether other constituents found in *P. edulis* BF, *i.e.* cyanogenic glycosides, can additively or synergistically enhance the anxiolytic effect has yet to be examined. Our results present further evidence for the efficacy and complementarity of UPLC-MS metabolomics in the field of drug discovery to speed up natural product research, thereby helping in reducing the gap that has opened to modern drug discovery demands because of too lengthy discovery and development times when starting from natural resources.

Abbreviations

Agl	Aglycon
AQF	Aqueous mother liquor fraction
BF	Butanol fraction
CF	Chloroform fraction
DZ	Diazepam
EAE	Ethyl acetate fraction
EE	Ethanol extract
GABA	γ -Aminobutyric acid
GABA _A R	GABA _A receptors
<i>P.</i>	<i>Passiflora</i>
PFF	Petroleum ether fraction
OE%	Percentage of open arms entries
OT%	Percentage of time spent in open arms

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