Pharmacognosy and Chemotypes of Passionflower (*Passiflora incarnata* L.)

Hans WOHLMUTH,^{*,a,b,c} Kerry George PENMAN,^d Tanya PEARSON,^c and Reginald Paul LEHMANN^d

^a Medicinal Plant Herbarium, Southern Cross University; ^b Centre for Phytochemistry and Pharmacology, Southern Cross University; ^c School of Health and Human Sciences, Southern Cross University; P.O. Box 157, Lismore NSW 2480, Australia; and ^d MediHerb Research Laboratories; Brisbane Technology Park, Eight Mile Plains QLD 4064, Australia.

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Passionflower (*Passiflora incarnata* L.) is used in phytotherapy as a mild sedative and anxiolytic agent. In the literature it is clear this plant shows considerable qualitative and quantitative variability with respect to its content of C-glycosyl flavones, some of which are used as marker compounds for extracts. Analysis of plant material cultivated in Australia revealed two chemically distinct groups; hence an investigation was carried out to determine whether distinct intraspecific chemotypes exist in this species. Eleven *P. incarnata* samples were analysed by HPLC, LC-MS and two different TLC methods. The samples fell into two distinct groups with respect to their C-glycosyl flavone profile, with little within-group variation. One chemotype was dominated by isovitexin and schaftoside/isoschaftoside, as is most widely reported in the literature for this species. The other chemotype was characterized by a high level of swertisin, with low levels of schaftoside/isoschaftoside. The two chemotypes are readily identified by both HPLC and TLC. Although the compounds responsible for the therapeutic activity of *P. incarnata* are yet to be identified, phytomedicines should be made with the accepted isovitexin chemotype until the pharmacological implications of chemotypical differences are understood.

Key words *Passiflora incarnata*; swertisin; herbal quality control; C-glycosyl flavone

The genus *Passiflora* L. comprises about 520 species of dicotyledonous plants in the family Passifloraceae.¹⁾ The majority of species are vines with most found in Central or South America and some species occurring in North America, Southeast Asia and Australia.²⁾ A number of species, including *P. edulis* SIMS, *P. laurifolia* L. and *P. mollissima* (KUNTH) BAILEY, are widely cultivated for their edible fruits, while many others are grown as ornamentals for their unusual and often spectacular flowers.¹⁾ Several species have a history of use as traditional herbal medicines,³⁾ and the North American passionflower *P. incarnata* L. is an important herbal drug widely used in contemporary Western phytotherapy. This species, commonly known as maypop, is native to the south-eastern United States, but is also cultivated in Europe, Asia and Australia, both as an ornamental and as a medicinal plant. *P. incarnata* is widely used in phytotherapy for its mild sedative and anxiolytic properties,^{4,5)} and the botanical drug (comprising the dried aerial parts of the plant) is included in the current European and British Pharmacopoeias.⁶⁾ Despite considerable work having been carried out, the active compounds responsible for the therapeutic properties of the plant have not been identified.^{7–9)}

P. incarnata is characterized phytochemically by a suite of C-glycosyl flavones (Fig. 1) such as vitexin (1), isovitexin (2), schaftoside (3), isoschaftoside (4) and isovitexin-2''-O-glucoside, although it is clear from the existing literature that considerable qualitative and quantitative variation occurs with respect to these flavonoids.^{10–15)}

During routine testing of samples of *P. incarnata* pharmaceutical raw material grown in Australia it became apparent that some of this material displayed a consistent flavonoid profile that was distinctly different from passionflower material obtained from other sources. While it has not been demonstrated that the flavonoids are directly linked with the drug's pharmacological activity,^{9,12)} these compounds are widely used as markers for authentication and quality purposes. Therefore, we undertook an investigation of the

	R ₁	R ₂	R ₃
vitexin (1)	H	OH	glucosyl
isovitexin (2)	glucosyl	OH	H
schaftoside (3)	glucosyl	OH	α-L-arabinopyranosyl
isoschaftoside (4)	α-L-arabinopyranosyl	OH	glucosyl
swertisin (5)	glucosyl	OCH ₃	H

Fig. 1. C-Glycosyl Flavones from *Passiflora incarnata*

observed phytochemical variability of *P. incarnata* raw materials.

Thus the aim of the study was to determine if *P. incarnata* raw materials produced by some growers in Australia belong to a distinct chemotype with a flavonoid profile different to that seen in typical pharmaceutical raw material from North America and elsewhere. TLC, HPLC with photodiode array detection and LC/MS were the analytical techniques employed.

MATERIALS AND METHODS

Plant Materials The samples analysed are listed in Table 1. All samples comprised dried plant material except Sample P7, which was a *P. incarnata* extract produced by Indena S.p.A. (Milan, Italy). Besides this sample, 10 other samples of *P. incarnata* were analysed, including 7 commercial samples obtained from raw materials suppliers and 3 samples collected by the authors from cultivated plants. All samples were identified by an experienced pharmacognosist (H. W.). Voucher specimens and samples were deposited in the Medicinal Plant Herbarium at Southern Cross University

* To whom correspondence should be addressed. e-mail: hans.wohlmuth@scu.edu.au

Table 1. List of Samples Analysed

Taxon	Sample ID	Origin	Voucher no.
<i>P. incarnata</i>	P1	Australia ^{a)}	NCMD05-029
<i>P. incarnata</i>	P2	India ^{a)}	NCMD05-030
<i>P. incarnata</i>	P6	Australia ^{a)}	NCMD05-022
<i>P. incarnata</i>	P7	Indena extract	Batch RP1786
<i>P. incarnata</i>	P8	Australia ^{a)}	NCMD05-031
<i>P. incarnata</i>	P9	Italy ^{a)}	NCMD05-032
<i>P. incarnata</i>	P10	Australia ^{a)}	NCMD05-033
<i>P. incarnata</i>	P11	Australia ^{a)}	NCMD05-034
<i>P. incarnata</i>	P13	Mudgeeraba, QLD, Australia	NCM05-109
<i>P. incarnata</i>	P15	Mullumbimby, NSW, Australia	NCM04-061
<i>P. incarnata</i> 'Alba'	P14	Lismore, NSW, Australia	NCM04-019

a) Commercial samples obtained from raw material suppliers.

(Table 1).

Extract Preparation All plant samples consisted of dried leaf, tendrils and stem (flower and fruit were excluded). For TLC analysis, 1 g of ground material was extracted in 10 ml methanol with 2×15-min sonication, filtered through a Millipore polyvinylidene fluoride (PVDF) filter into an amber vial and stored at 4 °C until analysis. For HPLC analysis, 1 g of ground material was extracted in 50 ml 70% ethanol with 2×15-min sonication, filtered through a Millipore PVDF filter into an HPLC snap-cap vial, sealed and stored at 4 °C until analysis.

TLC Methods Two TLC methods were compared. For both methods Sigma-Aldrich silica plates on aluminium with fluorescent indicator were used. Three reference standards were used: rutin (Sigma) 0.1%, chlorogenic acid (Sigma) 0.1% and hyperoside (quercetin-3-D-galactoside; Fluka) 0.1%, all in methanol. Ten microliters of samples and standards were applied to the plate in a band. All plates were viewed and photographed under UV 366 nm.

TLC Method 1: This was the method for Passion Flower in the British Pharmacopoeia (2007). Mobile phase was composed of water/anhydrous formic acid/methyl ethyl ketone/ethyl acetate (10/10/30/50 v/v), and the plates were developed to a distance of 150 mm at room temperature. Detection was by way of two spray reagents: Natural Products Reagent (2-aminoethyl diphenylborinate; Sigma; 1% in methanol) followed by Macrogol 400 (polyethylene glycol; Sigma; 5% in methanol).

TLC Method 2: This method was developed by Widmer, Meier and Schaffner and was published by CAMAG, Switzerland, on their website (<http://www.camag.com/index.php>; n.d.). Mobile phase was composed of tetrahydrofuran/toluene/formic acid/water (16/8/2/1 v/v), and the plates were developed to a distance of 52 mm at room temperature. The heated plates were sprayed with Natural Products Reagent (0.5% in ethyl acetate) followed by polyethylene glycol 4000 (Fluka; 5% in dichloromethane).

HPLC Method Reverse-phase HPLC analysis was performed on a Shimadzu VP series LC-10Avp HPLC configured as a quaternary low-pressure gradient mixing setup. The system was equipped with a SIL-10ADvp autoinjector, CTO-10ACvp column oven, LC-10ATvp pump, SPD-M10Avp photodiode array detector and SCL-10Avp system controller.

The software was VP v. 6.12 SP2. Mobile phase C was 50 mM phosphoric acid in water, mobile phase D was acetonitrile. The gradient profile was C/D (94/6, v/v) to C/D (69.6/30.4) over 14 min, to C/D (0/100) over 11 min, to C/D (94/6) over 6 min, then C/D (94/6) for 8 min. Mobile phase was pumped at 0.8 ml/min, the column temperature was 45 °C, and the injection volume was 10 µl. Data were collected using a UV-VIS photodiode array detector collecting absorption spectra from 200 to 400 nm with quantification performed at 340 and 240 nm. Vitexin (Chromadex, Irvine, CA, U.S.A.) was used as a standard.

LC/MS Method LC/MS analysis was carried out on a Shimadzu VP series LC-10Avp HPLC configured for high-pressure gradient solvent mixing. The system was equipped with a SIL-10 ADvp autoinjector, CTO-10ACvp column oven, 2 LC-10ADvp pumps, SPD-M10Avp photodiode array and a SCL-10Avp system controller interfaced to either a Shimadzu LCMS-QP8000 Mass Spectrometer (LCMS Solutions v. 2.00 Su1B) or a Shimadzu LCMS-2010EV Liquid Chromatograph Mass Spectrometer (LCMS Solutions v. 3.40.299). Mobile phase A was water (Millipore Rios/Synergy Systems), mobile phase B was acetonitrile. The gradient profile was A/B (95/5) to A/B (50/50) over 30 min, held at A/B (50/50) for 10 min, returned to A/B (95/5) over 5 min with a 10 min re-equilibration time. Flow rate was 0.3 ml/min. Mass spectral data were acquired in both positive and negative ion modes on the APCI probe which was set at 400 °C with the CDL interface at 250 °C (for the LCMS 2010EV setup the heater block was set to 200 °C). Corona needle was at 4.5 kV and the detector voltage set to 1.45 V.

Assignment of schaftoside/isoschaftoside, isovitexin, vitexin, swertisin and isovitexin-2-O-β-glucopyranose was made using the LC, UV and mass spectrometric data in conjunction with the published literature.

RESULTS

The UV spectra obtained by the photodiode array detector readily distinguished two sets of flavonoids in *P. incarnata*. The major group of flavonoids found to be present is derived from an apigenin (4',5,7-trihydroxyflavone) base structure, while a few are derived from luteolin (3',4',5,7-tetrahydroxyflavone). Standard rules on the effects of substituents on absorption maxima were used to interpret the UV-VIS data. Several examples of a $\lambda_{\max}$ shifting from 336 nm to approximately 348 nm were observed; this is consistent with the addition of a hydroxyl group. Mass spectroscopic data readily yielded parent ions and fragmentation patterns that were matched to the flavonoid structures. Observed fragmentation patterns were correlated to the loss of various sugar moieties. The vitexin reference standard was readily matched to the vitexin in the samples.

HPLC and LC/MS analyses of the 11 samples of *P. incarnata* showed that they fell into two distinct groups with respect to their C-glycosyl flavone profile (retention time 22–25 min by LC-MS). The high degree of consistency in flavonoid profile across samples of different origin in each of the two groups warrants the designation of these as distinct chemotypes.

Samples cultivated in India (P2), Italy (P9) and two cultivated in Australia (P13 and P15) contained isovitexin (**2**) and

Fig. 2. HPLC Chromatogram Overlay of Four *P. incarnata* Samples of the Isovitexin Chemotype

Top to bottom: P9, P7, P2, P13. P7 is a commercial Indena extract.

Fig. 3. HPLC Chromatogram Overlay of Six *P. incarnata* Samples of the Swertisin Chemotype

Top to bottom: P11, P10, P8, P6, P1, P14.

schaftoside (3)/isoschaftoside (4) as major flavonoids with little or no swertisin (5) present. The flavonoid profiles of these samples were highly consistent and virtually identical to that of the passionflower extract from a leading extract manufacturer, Indena S.p.A. (Fig. 2).

The other 6 samples displayed a very different but highly consistent flavonoid profile with swertisin (5) being the predominant flavonoid and schaftoside (3) and isoschaftoside (4) occurring at very low levels (Fig. 3). At least one of the samples of swertisin chemotype was from the white flowered cultivar, *P. incarnata* 'Alba,' which is cultivated by some Australian growers.

Both chemotypes contained vitexin (1) and isovitexin (2), but samples of the schaftoside/isoschaftoside chemotype contained significantly more isovitexin than vitexin, unlike the samples of the swertisin chemotype.

We found TLC Method 2 to be superior to the method given in the British Pharmacopoeia (Method 1) in terms of clarity and resolution (results not shown), and Method 2 allowed for the differentiation between the two *P. incarnata* chemotypes (Fig. 4).

DISCUSSION

Variability in the C-glycosyl flavone content of *P. incarnata* is well documented, but distinct chemotypes have not previously been described for this species. Rehwald and colleagues analysed 14 *P. incarnata* samples obtained commercially in Switzerland and Germany and found the chromatograms showed no uniformity. The major compound(s) were isovitexin in five samples, isovitexin-2''-O-glucoside in

Fig. 4. TLC Plate Showing *P. incarnata* Swertisin Chemotype (1–6) and Isovitexin Chemotype (7, 8)

C=chlorogenic acid, H=hyperoside, R=rutin.

four samples, and schaftoside and isoschaftoside in three samples; swertisin was the major flavonoid in two of the samples.¹⁴ An Italian group reported schaftoside, isoschaftoside and isovitexin-2''-O-β-glucopyranoside as the major flavonoids of *P. incarnata* extracts of undisclosed origin.¹³

A French group analysed 18 commercial samples of *P. incarnata* originating from Europe, India and U.S.A. by capillary zone electrophoresis and found them falling into five groups: Group I (13 samples) was characterized by a high content of isovitexin, Group II (two samples) by swertisin, Group III (two samples) by isovitexin 2''-O-glucoside, whereas Group IV (one sample) had four main compounds (isovitexin, schaftoside, isoschaftoside and isoorientin).¹⁵ Groups I–III corresponded well to samples described by Rehwald's group. All the European samples fell into Group I (isovitexin), while four samples from the U.S.A. fell into the four different groups. In another study, one sample was dominated by schaftoside and isoschaftoside, another by isovitexin.⁷

A group from the University of Vienna analysed 10 commercial samples of *P. incarnata* obtained from European suppliers by capillary electrophoresis.¹⁰ Seven of these samples had isovitexin-2''-O-glucopyranoside as the major compound, two had isovitexin and one sample had swertisin as major flavonoids.

Based on the literature cited above and the results of the present study, it is clear that *P. incarnata* extracts display very considerable variability with respect to their C-glycosyl flavone profiles. It is evident from these studies, however, that most commercial samples are dominated by isovitexin, while only a few have swertisin as the major flavonoid.

Given the significant degree of variability reported in the literature, it is somewhat surprising that the 11 samples analysed by us fell into only two groups, each showing little variability. In terms of the six Australian samples of the swertisin chemotype, it is theoretically possible that they all could have originated from plants with a sole ancestor in the form of seed imported to Australia from North America several decades ago. A similar explanation is not possible for the samples of schaftoside/isoschaftoside chemotype, as they were sourced from three different continents.

In conclusion, the findings of this study demonstrate the existence of a second distinct chemotype of *P. incarnata* characterised by a very high swertisin content and the virtual absence of schaftoside/isoschaftoside. This chemotype has a flavonoid profile that is distinctly different from typical *P. incarnata* material used in the pharmaceutical industry, which is characterised by a high content of isovitexin and schaftoside/isoschaftoside, and the absence (or virtual

absence) of swertisin. Swertisin has been shown to possess antioxidant and alpha-glucosidase inhibitory activity,^{16,17)} but little else is known about the pharmacological actions of this flavonoid.

Although the pharmacological activity of *P. incarnata* has not been linked to specific flavonoid compounds, the existence of chemotypes within the species raises quality issues for the herbal medicine and phytopharmaceutical industries. Until the potential pharmacological and therapeutic implications of chemotypical differences are understood, phytomedicines containing *P. incarnata* should be prepared using plant material characterised by the typical flavonoid profile showing little or no swertisin.

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