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**Reference:**
Zarev Yancho, Popova Pavlinka, Foubert Kenn, Ionkova Iliana, Pieters Luc.- Comparative LC–MS analysis of tropolone alkaloids from in vitro cultures and native sources of Gloriosa superba by Kendrick mass defect plots
Phytochemical analysis - ISSN 0958-0344 - Hoboken, Wiley, 32:4(2021), p. 446-456
Full text (Publisher's DOI): https://doi.org/10.1002/PCA.2992
To cite this reference: https://hdl.handle.net/10067/1711090151162165141

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# Comparative LC-MS analysis of tropolone alkaloids from *in vitro* cultures and native sources of *Gloriosa superba* by Kendrick mass defect plots

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**Acknowledgments**

The Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students" supported this work for research.

20

21 **ABSTRACT:**

22 **Introduction** - *Gloriosa superba* L. is a promising antitumoural plant species as a source of 23 colchicinoids. Ethnobotanical applications of *G. superba* are associated with different plant parts 24 such as leaves, seeds, fruits, tuber and the whole plant.

25 **Objectives** – A comparative phytochemical study of purified extracts from *in vitro* cultures and 26 native tubers of *G. superba* was carried out by ultra-high-performance liquid chromatography-27 high-resolution mass spectrometry (UHPLC-HR-MS) in combination with the mass defect 28 filtering (MDF) technique.

29 **Material and methods** - The individual compounds were tentatively annotated using database 30 correlations, retention time (*Rt*), accurate *m/z* data obtained by (ESI) (+)-HR-MS, proposed 31 elemental composition, ring double bond equivalent (RDBeq) values and HR-MS/MS 32 fragmentation patterns. Moreover, the identification was based on transforming the exact mass 33 ratio (*m/z*) for the protonated molecular ions $[M+H]^+$ of the observed metabolites in Kendrick 34 nominal masses (NKM) and calculation of the Kendrick mass defect (KMD), which made it 35 possible to graphically present the ion peaks in Kendrick plots.

36 **Results** - Building Kendrick plots allows easy differentiation of small structural differences such 37 as methylation or demethylation of compounds from the same homologous series. In this way, a 38 wide range of tropolone alkaloids was characterized. A greater variety was observed in *in vitro* 39 cultures, compared to native sources.

40 **Conclusion** - This LC-MS analysis unambiguously demonstrated the presence of tropolone 41 alkaloids in *in vitro* cultures of *G. superba*. This approach of LC-MS data interpretation can be 42 used to understand complex mass spectra such as those of plant extracts.

43 **Keywords**

44 *Gloriosa superba*, Kendrick mass defect, comparative LC-MS analysis, colchicinoids, alkaloids

45

46 **INTRODUCTION**

47 *Gloriosa superba* L. is perennial medicinal plant belonging to the family Liliaceae. It is widely
48 distributed throughout India, but also native to tropical Africa. (Maroyi and Van der Maesen,
49 2011). *G. superba* is rich in colchicinoids, alkaloids belonging to the group of colchicine binding
50 site tubulin inhibitors (CBSIs). The trimethoxyphenyl group is most important for modulating
51 the pharmacological properties (Li et al., 2017). Nowadays, there is increasing interest in
52 glycoconjugates, such as colchicoside, which is considered less toxic than colchicine (Capistrano
53 et al., 2016a). In addition to the known glycosylated colchicine derivatives, such as colchicoside
54 (Mimaki et al., 1991), its isomer 3-*O*-demethylcolchicine-3-*O*-*α*-D-glucopyranoside (Suri et al.,
55 2001), and colchicodiside C, which was reported previously as a biotechnologically-derived
56 product, four novel colchicinoids named glorioside, colchicodiside A, gloriodiside and
57 colchicodiside B were identified more recently in *G. superba* (Zarev et al., 2017). From the
58 seedless pod of *G. superba* of Thai origin, three novel glycosylated colchicinoids -
59 dongduengoside A-C were isolated, together with colchicine, 2-demethylcolchicine and
60 colchicoside (Sahakitpichan et al., 2016).

61 The ethnobotanical applications of *G. superba* are associated with five different plant parts,
62 including the leaves, seeds, fruits, tuber and the whole plant. The tuber is most frequently used
63 one as a germicide, to cure ulcers, leprosy, scrofula, worm infections, hemorrhoids, debilitating
64 arthritis, snake bites, etc. Capistrano et al., 2016b carried out a survival experiment in a murine
65 model of pancreatic adenocarcinoma induced by PANC02 cells. Slightly longer survival was
66 observed for the group treated with a colchicoside rich extract, while combinatory treatment of
67 total seed extract of *G. superba* with gemcitabine demonstrated a significant effect on tumour
68 growth.

69 Apart from native sources, also *in vitro* plant cell cultures can be used for the production of
70 biologically active plant extracts. Herein we present an approach for the interpretation of
71 complex LC-MS data of different plant sources, including *in vitro* shoot cultures and *in vitro*
72 tubers, as well as native tubers of *G. superba*, which resulted in the characterization of a series of
73 colchicinoids.

74 **EXPERIMENTAL**

75 **Plant material**

76 The *in vitro* plant material of *G. superba* was obtained from shoot cultures and tubers,
77 successfully grown in the lab (Zarev and Ionkova, 2013). The native tubers of *G. superba* were
78 provided by National Botanical Garden in Meise, Belgium.

79 **Chemicals and reagents**

80 Solvents used for LC-MS analysis, i.e. acetonitrile and water (LC-MS grade), as well as ethanol,
81 methanol and *n*-hexane (HPLC-quality), were purchased from Fisher Chemicals (Loughborough,
82 UK). The colchicine standard was obtained from Acros Organics (Geel, Belgium).

83 **Extraction procedure and sample preparation**

84 Well-dried plant material from the various *in vitro* cultures (1 g) and native tubers of *G. superba*
85 was extracted with 70% ethanol (2 x 50 mL) in an ultrasonic bath (35 kHz) for 40 min adding
86 each time a fresh portion of solvent. The extracts were filtered through a paper filter and
87 concentrated using a rotary vacuum evaporator, and then defatted with *n*-hexane (3 x 60 mL).
88 The defatted extract was purified by open column chromatography (15 x 350 mm) with MCI gel
89 (8 g) (CHP20P, 75-150 μm particle size, Mitsubishi Chemical Co., Japan). The column was
90 eluted with water and increasing concentrations of methanol (20%, 40%, 60%, 80% and 100%).
91 Each fraction of 30 mL was eluted in 50 mL volumetric flasks, and set to the mark with 50%

92 methanol. The obtained fractions were transparent, non-palliating solutions, from which a 1 mL
93 aliquot was used for LC-MS analysis.

94 **LC-HRMS analysis**

95 UHPLC-HRMS analysis was performed using a Thermo Scientific Dionex Ultimate 3000 RSLC
96 instrument (ThermoFisher Scientific, Germering, Germany) consisting of a six-channel degasser
97 SRD-3600, a high-pressure gradient pump HPG-3400RS, an autosampler WPS-3000TRS and a
98 column compartment TCC-3000RS coupled to a Thermo Scientific Q Exactive Plus mass
99 spectrometer (ThermoFisher Scientific, Inc., Bremen, Germany). UHPLC separations were
100 performed on a Kinetex C18 column (Phenomenex Corporation, Torrence, CA, USA) (2.1 x 100
101 mm, 2.6 μm) at 40 °C. Each chromatographic run was carried out with a binary mobile phase
102 consisting of water containing 0.1% (v/v) formic acid (A) and acetonitrile also with 0.1% (v/v)
103 formic acid (B). A gradient program was performed as follows: 0-3 min, 10% B; 3-23 min, 10-
104 70% B; 23–25 min, 95% B. The flow rate was 0.3 mL/min, and the sample injection volume was
105 1 μL. Full scan data were recorded in (+) ESI mode from *m/z* 250 to 1 000 at a resolution of 70
106 000 (at *m/z* 200). Full scan dd-MS/MS (top 5) was performed at a resolution of 17 500 (at *m/z*
107 200), AGS target 1e5 with maximum IT 50 ms. Xcalibur software ver. 4.0 was used for data
108 acquisition and processing.

109 **LC-HRMS data processing and Kendrick mass defect analysis**

110 The concept of a mass defect arises from the fact that each isotope of a chemical element
111 releases a different amount of energy in the formation and stabilization of its nucleus. The mass
112 defect of a single element or chemical compound is calculated as the difference between the
113 exact mass of the isotope and its nominal mass (the number of protons and neutrons in a given
114 formula). Modern high-resolution mass-spectrometry relies on the mass defect to measure the

115 exact masses of the formed ions. The fact that each molecule has its own unique mass allows to
116 clarify the chemical formulas of unknown compounds, as well as to confirm the formulas of
117 known compounds with great precision.
118 Kendrick mass defect analysis is a method that can be used to understand complex mass spectra
119 such as those obtained from plant extracts. With the Kendrick mass defect, it becomes possible
120 to distinguish compounds in a complex mixture. Since mass defect is associated with the binding
121 energy of the nucleus, most compounds have a negative mass defect because the nuclear power
122 increases with increasing of the atomic numbers. Hydrogen has the most significant positive
123 mass defect (7.825 mDa) and therefore it has a significant contribution to the mass defect of any
124 naturally occurring metabolite (Pourshahian, 2017; Sleno, 2012). Working on complex mass
125 spectra can be facilitated by building a worktop in which the nominal masses (with a difference
126 of 1 Da) are applied to the x-ordinate, and the individual mass defect for each observed
127 molecular ion (with exactly obtained *m/z*) to the y-ordinate. In this case, the mass defect is the
128 difference between the actual mass (produced during the analysis) and the nominal mass.
129 Protonated molecular ions with *m/z* values, which differ only by the exact mass of a particular
130 functional group, e.g. $CH_2$, form homologous series. For hydrocarbon systems, the IUPAC mass
131 can be converted to the Kendrick mass by multiplying the ratio of the nominal mass to the exact
132 mass (14.00000/14.01565) with the *m/z* value of the protonated ion as shown in the equation
133 below:

134 Kendrick mass = Exact mass *m/z* of the observed peak x 14.0000/14.0157

135 Then KMD is calculated as the Kendrick mass subtracted from the nominal mass:

136 KMD = the nominal mass - the Kendrick mass

137 Thus, ions differing by only one $CH_2$ group (e.g., methylation) will have the same KMD value,
138 i.e. they will form a homologous series. When a Kendrick mass defect plot is constructed (KMD
139 against the Kendrick nominal mass), each homologous series coincides in a horizontal line.
140 Depending on the structure of the studied molecular ions, the construction of Kendrick mass
141 defect plots may be more appropriate using other functional groups ($OCH_2$, COO, CO, etc.)
142 (Kendrick, 1963).

143 Since the focus was on tropolone alkaloids, containing a nitrogen atom, protonated molecular
144 ions with even values were of interest. For reducing the number of possible elemental
145 composition (EC) candidates, mass tolerance was set at 3 ppm, and 5 ppm in case of fragments.
146 C, H, O and N were set for elemental composition and only adequate RDBeq values were
147 considered. Fragmentation pattern and retention time of colchicine were compared with a
148 colchicine reference. MS/MS spectra of the other metabolites were manually inspected and
149 compared with the METLIN database (Smith et al., 2005).

150 **RESULTS AND DISCUSSION**

151 UHPLC-HR-MS was used to compare the metabolomic profile of samples from *in vitro* cultures
152 and native tubers of *G. superba*. Results are presented in Table 1. The main metabolite,
153 colchicine (**1**) (Figure 1) was observed in positive ion mode as a protonated molecular ion with
154 *m/z* 400.1758 $[M+H]^+$ and corresponding molecular formula $C_{22}H_{26}NO_6$, eluted at *Rt* 10.05 min.
155 Its MS/MS spectrum gave seven fragment ions. One of the rather abundant ions common for the
156 whole range of analyzed compounds is produced through the loss of water $[M+H-H_2O]^+$ (-18
157 Da). Other decomposition reactions included the loss of an acetamide molecule $NH_2COCH_3$ (-59
158 Da), as well as the ketene $CH_2=C=O$ (-42 Da), which is typical for molecules with an acetylated
159 $-NH_2$ group. Loss of a $CH_3O$ group of colchicine results in a $[M+H-CH_3OH]^+$ (-32 Da)

160 fragment. Dissociation of the parent ion leading to the fragment ion with *m/z* 324.12 involves
161 radical cleavage, consisting of combined elimination of $NH_2COCH_3$, with CO losses (-76 Da).
162 The presence of a fragment ion at *m/z* 326.15 (-74 Da), which is formed from combined loss of
163 ketene ($CH_2$=C=O) and methanol ($CH_3OH$) molecules, shows that the $CH_3O$ moiety originates
164 from the substituent in position 10 of colchicine and not from the methoxy groups attached to the
165 six-membered aromatic ring (Table 1) (Kurek et al., 2015). After transforming the MS data and
166 building up the Kendrick plot (Figure 2), the formation of several homologous series of
167 precursor ions is clearly visible. The colchicine precursor ion is part of a homologous series (**a**)
168 along with protonated pseudomolecular ions $[M+H]^+$ with *m/z* 386.1599, 372.1441 and
169 358.1284, corresponding to the molecular formulas $C_{21}H_{24}NO_6$, $C_{20}H_{22}NO_6$ and $C_{19}H_{20}NO_6$,
170 respectively, which differ by one $CH_2$ group. Compounds (**2**-**7**) have an identical precursor ion
171 *m/z* 386.1599 and identical molecular formula $C_{21}H_{24}NO_6$, which unambiguously shows the
172 presence of isomers (Table 1). Analyzing the main fragments in the MS/MS spectrum and
173 comparing those values with the Metlin database led to the tentative characterization of the
174 following metabolites: (**3**, **5**) with the main fragment ions at *m/z* 368.15, 354.13, 344.15 and
175 327.12 (3-demethylcochicine or its positional isomer 2-demethylcochicine) and (**4**), which
176 differs from **3** and **5** only by *Rt* and fragmentation pattern (main fragment ion at *m/z* 344.15,
177 described as an unknown compound); (**7**) with main fragment ions at *m/z* 368.15 and *m/z* 310.12
178 (colchiceine); (**6**) with the main fragment ion at *m/z* 327.12 (gloriosine) and (**2**), which differ
179 from (**6**) only by its *Rt*, also described as unknown compound. Compounds (**8**-**10**) have an
180 identical precursor ion with *m/z* 372.1441 and an identical molecular formula $C_{21}H_{24}NO_6$, which
181 are part from the same homologous series (**a**), thus they differ from compounds (**2**-**7**) by one $CH_2$
182 group. The lack of fragment ions typical for an acetylated primary amino-group (-42 Da and -59

183 Da) in the MS/MS spectrum of compound **10** is indicative for the presence of an *N*-formyl group.
184 The fragment ion resulting from the typical methoxylation at C-10 (-32 Da) is also missing.
185 Thus, the proposed structure for compound **10** is *N*-deacetyl-*N*-formylcolchiceine (Figure 1). The
186 MS/MS spectra of compounds **8** and **9** are rather similar and yielded product ions at *m/z* 330.13
187 and 313.11, which are the result of the cleavage of an acetyl group. The product ion at *m/z*
188 340.12 $[M+H-CH_3OH]^+$ of compound **8** corresponds to methoxylation at C-10 in the
189 colchicinoid skeleton. Nevertheless, the position of de-methylation could not be strictly defined,
190 thus, compound **8** was tentatively identified as 1,2-didemethylcolchicine or 2,3-
191 didemethylcolchicine and compound **9** as 3-demethylcolchiceine or 2-demethylcolchiceine
192 (Figure 1). Whereas compounds **1** – **10** correspond to colchicinoids that have been reported
193 before or their isomers, to the best of our knowledge a constituent with a protonated molecular
194 formula $C_{19}H_{20}NO_6$ (*m/z* 358.1284, *Rt* 5.84 min) (**11**) has not been described before; most likely
195 it is a new natural compound with three $CH_2$ groups less than colchicine. From the product ions
196 in the MS/MS spectrum of compound **11** it seems that it concerns an *N*-formyl derivative without
197 methylation at C-10, and with a mono de-methylation in position 1, 2 or 3.
198 Figure 2 also clearly shows the homologous series (**c**), where a protonated molecular ion with
199 *m/z* 370.1283 differs from those with *m/z* 384.1442 only by one $CH_2$, which is also visible in the
200 corresponding molecular formulas of the observed ions $C_{20}H_{20}NO_6$ and $C_{21}H_{22}NO_6$, respectively.
201 The RDBeq value for the both ions is 12, which is suggesting the presence of four rings in the
202 molecule, while a monosaccharide moiety attached to tropolone skeleton is lacking. Thus, the
203 protonated molecular ion (**12**) with *m/z* 384.1442 $[M+H]^+$ is defined as cornigerine (Figure 1),
204 containing a methylenedioxy bridge. The protonated molecular ion (**13**) with *m/z* 370.1283
205 $[M+H]^+$ is a derivate containing one $CH_2$ group less. After literature review, it appeared that the

206 protonated molecular ion (**13**) with *m/z* 370.1283 relates to a demethylated derivative of
207 cornigerine that has not yet been described.
208 When analyzing the Kendrick plots shown in Figure S1 it can be observed that protonated
209 molecular ions with *m/z* 386.1599 $[M+H]^+$ and *m/z* 400.1758 $[M+H]^+$ are different from those
210 with *m/z* 344.1497 and 358.1652 by a $C_2H_2O$ moiety (acetyl group) with KMD -0.062 and -
211 0.074, respectively. On the other hand, protonated molecular ions $[M+H]^+$ with *m/z* 344.1497
212 and 358.1652 along with *m/z* 372.1804 and *m/z* 386.1964 with corresponding molecular formulas
213 $C_{19}H_{22}NO_5$, $C_{20}H_{24}NO_5$, $C_{21}H_{26}NO_5$ and $C_{22}H_{28}NO_5$, respectively, are part of their own
214 homologous series (**b**), where the described molecules are differing just by one $CH_2$ group
215 (Figure 2). Following this analysis and comparing the main fragments after MS/MS
216 fragmentation with the Metlin database and literature sources, we are suggesting from
217 homologous series (**b**) the presence of the following metabolites: *N*-deacetylcolchiceine (**14**), *N*-
218 deacetyl-colchicine (**15**) with main fragment ions *m/z* 341.14, 326.15, demecolcine (**16**) with
219 main fragment ions *m/z* 341.14, 326.15, (colchiceine, 7-deacetyl-dimethyl) (**17**) and *N*-
220 methyldemecolcine (**18**) (Table 1, Figure 1). The putative structure for compound **19** with *m/z*
221 402.1550 and molecular formula $C_{21}H_{24}NO_7$ is gloriosamine D, which has an additional
222 hydroxyl group in position 6 compared to gloriosine (**6**) (Kitajima et al., 2008). This assignment
223 is also confirmed by the Kendrick plot shown in Figure S2 where it is clearly visible that **19**
224 makes a homologous series along with the protonated molecular ion with *m/z* 386.1599, differing
225 from each other by one oxygen atom. Compared to the described aglycones, several protonated
226 molecular ions demonstrated an additional RDBeq, which may be due to the additional ring
227 present in glycosides. This was confirmed by the MS/MS spectra, which unambiguously showed

228 a loss of 162 Da $[M+H-C_6H_{10}O_5]^+$, i.e. one hexosyl unit, in compounds **20-41**, corresponding to
229 mono-substituted *O*-glycosylated colchicinoids (Table 2).
230 Comparing the results obtained for the *in vitro* shoot cultures, *in vitro* tubers and native tubers, it
231 could be observed that colchicine (**1**) was always present. This was also the case for colchicoside
232 (**27**), the major glycoside from *G. superba*, as well as for compounds **3**, **8** and **16**; and from the
233 other glycosides compounds **22** and **29**. Only 3 compounds (i.e. **9**, **17** and **41**) were unique for
234 the native tubers, indicating the broad producing capacity, at least qualitatively, of the *in vitro*
235 cultures. A few compounds were unique for the *in vitro* shoot cultures; it can be noted that many
236 of the observed glycosides were unique for the *in vitro* tubers. The tentative newly described
237 compound **11** was only detected *in vitro*, in the shoot cultures as well as in the *in vitro* tubers. A
238 further notable observation was that in the native tubers 11 different compounds were detected (7
239 aglycones and 4 glycosides), in the *in vitro* tubers 32 (12 aglycones and 20 glycosides), and in
240 the *in vitro* shoot cultures 17 (10 aglycones and 7 glycosides), i.e. *in vitro* a wider variation was
241 observed than in the native tubers, mainly due to the range of different glycosides in the *in vitro*
242 tubers.
243 Our considerations for the suggested structures are based on careful analysis of the complex data.
244 Identifying metabolites based on mass spectrometry only could not be exhaustive and
245 unambiguous, because of the presence of many isomers and their glycosides. The identification
246 was based on retention time (*Rt*), accurate *m/z* data obtained by (ESI) (+)-HR-MS, proposed
247 elemental composition, ring double bond equivalent (RDBeq) values, HR-MS/MS data, and by
248 transforming the exact mass ratio or mass to charge ratio (*m/z*) for the protonated molecular ions
249 $[M+H]^+$ of the observed metabolites in Kendrick nominal masses (NKM) and calculation of the
250 Kendrick mass defect (KMD). In this way it was possible to graphically present the ion peaks in

251 Kendrick plots, in order to observe homologous groups differing only by the number of specific
252 structural units ($CH_2$, O, $C_2H_2O$ etc.). This approach unambiguously demonstrated the presence
253 of a wide range of tropolone alkaloids in *in vitro* cultures of *G. superba*. Predicted structures are
254 found in greater variety in *in vitro* cultures, compared to native sources.

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**Table 1.** Secondary metabolites identified in *in vitro* shoot and tuber as well as native tubers of *Gloriosa superba*. *Rt* (min), $[M+H]^+$ measured *m/z*, $[M+H]^+$ theoretical *m/z*, elemental composition (EC) (δ ppm), ring double bond equivalents (RDBeq).

| No | *Rt* (min) | $[M+H]^+$ meas. *m/z* | $[M+H]^+$ theor. *m/z* | DBE | EC (δ ppm) | MS/MS fragments | Fractions derived after MCI gel purification (% MeOH for elution) | | | | | | | | | | | Compounds |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *In vitro* shoot cultures | | | *In vitro* tuber | | | | native tubers | | | | |
| | | | | | | | 60% | 80% | 100% | 40% | 60% | 80% | 100% | 40% | 60% | 80% | 100% | |
| **1** | 10.06 | 400.1758 | 400.1755 | 10.5 | $C_{22}H_{26}NO_6$ (0.79) | 382.17 $[M+H-H_2O]^+$<br>368.15 $[M+H-CH_3OH]^+$<br>358.16 $[M+H-C_2H_2O]^+$<br>341.14 $[M+H-NH_2COCH_3]^+$<br>326.15 $[M+H-C_2H_2O-CH_3OH]^+$<br>324.12 $[M+H-NH_2COCH_3-CO]^+$ | + | + | + | | + | + | + | | | + | + | colchicine* |
| **2** | 6.47 | 386.1599 | 386.1598 | 10.5 | $C_{21}H_{24}NO_6$ (-0.78) | 354.13 $[M+H-CH_3O]^+$<br>327.12 $[M+H-NH_2COCH_3]^+$ | | | | | + | | | | | | | unknown |
| **3** | 7.65 | 386.1590 | 386.1598 | 10.5 | $C_{21}H_{24}NO_6$ (0.35) | 368.15 $[M+H-H_2O]^+$<br>354.13 $[M+H-CH_3OH]^+$<br>344.15 $[M+H-C_2H_2O]^+$<br>327.12 $[M+H-NH_2COCH_3]^+$ | | + | | | | + | + | | | + | + | 3-demethylcolchicine or 2-demethylcolchicine |
| **4** | 7.93 | 386.1599 | 386.1598 | 10.5 | $C_{21}H_{24}NO_6$ (0.25) | 344.15 $[M+H-C_2H_2O]^+$ | | + | | | | | | | | | | unknown |
| **5** | 8.23 | 386.1590 | 386.1598 | 10.5 | $C_{21}H_{24}NO_6$ (0.02) | 368.15 $[M+H-H_2O]^+$<br>353.13 $[M+H-CH_3OH-H]^+$<br>344.15 $[M+H-C_2H_2O]^+$<br>327.12 $[M+H-NH_2COCH_3]^+$ | | + | | | | | | | | | | 3-demethylcolchicine or 2-demethylcolchicine |
| **6** | 9.12 | 386.1598 | 386.1598 | 10.5 | $C_{21}H_{24}NO_6$ (-1.56) | 327.12 $[M+H-NH_2COCH_3]^+$ | | + | + | | | + | + | | | | | gloriosine |
| **7** | 10.00 | 386.1599 | 386.1598 | 10.5 | $C_{21}H_{24}NO_6$ (-1.25) | 368.15 $[M+H-H_2O]^+$ 353.13 $[M+H-CH_3O-H]^+$ 310.12 $[M+H-NH_2COCH_3-CO]^+$ | | + | + | | | + | + | | | | | colchiceine |
| **8** | 5.95 | 372.1441 | 372.1447 | 10.5 | $C_{20}H_{22}NO_6$ (-1.57) | 354.13 $[M+H-H_2O]^+$ | | + | | | + | | | | + | + | | 1,2-didemethylcolchicine |

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 340.12 [M+H-$CH_3OH$]$^+$<br>330.13 [M+H-$C_2H_2O$]$^+$<br>313.11 [M+H-$NH_2COCH_3$]$^+$ | | | | | | | | | | | | or 2,3-didemethylcolchicine |
| **9** | 6.77 | 372.1441 | 372.1447 | 10.5 | $C_{20}H_{22}NO_6$ (-1.38) | 354.135 [M+H-$H_2O$]$^+$<br>330.13 [M+H-$C_2H_2O$]$^+$<br>313.11 [M+H-$NH_2COCH_3$]$^+$ | | | | | | | | | + | + | | 3-demethylcolchiceine or 2-demethylcolchiceine |
| **10** | 7.62 | 372.1443 | 372.1447 | 10.5 | $C_{20}H_{22}NO_6$ (-1.22) | 354.13 [M+H-$H_2O$]$^+$<br>339.11 [M+H-$CH_3OH$-H]$^+$ | | + | | | | | | | | | | *N*-deacetyl-*N*-formylcolchiceine |
| **11** | 5.84 | 358.1284 | 358.1291 | 10.5 | $C_{19}H_{20}NO_6$ (-1.63) | 340.12 [M+H-$H_2O$]$^+$<br>325.09 [M+H-$CH_3OH$-H]$^+$ | + | | | | + | + | | | | | | unknown |
| **12** | 10.94 | 384.1442 | 384.1442 | 11.5 | $C_{21}H_{22}NO_6$ (-1.18) | 366.13 [M+H-$H_2O$]$^+$<br>342.13 [M+H-$C_2H_2O$]$^+$<br>325.11 [M+H-$NH_2COCH_3$]$^+$ | | | | | | | + | | | + | + | cornigerine |
| **13** | 5.81 | 370.1283 | 370.1285 | 11.5 | $C_{20}H_{20}NO_6$ (-0.50) | 328.12 [M+H-$C_2H_2O$]$^+$<br>311.09 [M+H-$NH_2COCH_3$]$^+$ | | + | | | | | | | | | | unknown |
| **14** | 5.55 | 344.1497 | 344.1498 | 9.5 | $C_{19}H_{22}NO_5$ (-0.40) | | | | | | + | | | | | | | *N*-Deacetylcolchiceine (trimethylcolchicinic acid) |
| **15** | 7.36 | 358.1652 | 358.1655 | 9.5 | $C_{20}H_{24}NO_5$ (-0.47) | | | | | | + | + | | | + | | | *N*-deacetylcolchicine |
| **16** | 5.36 | 372.1812 | 372.1811 | 9.5 | $C_{21}H_{26}NO_5$ (-2.14) | | + | | | + | + | | | + | | | | demecolcine (colcemide, *N*-methylcolchamine) |
| **17** | 6.02 | 372.1804 | 372.1811 | 9.5 | $C_{21}H_{26}NO_5$ (-1.82) | | | | | | | | | + | | | | colchiceine, 7-deaceto-demethyl |
| **18** | 7.78 | 386.1964 | 386.1968 | 9.5 | $C_{22}H_{28}NO_5$ (-0.95) | | | | | | + | | | | | | | *N*-methyldemecolcine |
| **19** | 6.27 | 402.1550 | 402.1558 | 10.5 | $C_{21}H_{24}NO_7$ (-0.39) | | | | | | + | | | | + | | | gloriosamine D |

**Table 2.** Glycosylated secondary metabolites identified in *in vitro* shoot and tuber as well as native tubers of *Gloriosa superba*. *Rt* (min), $[M+H]^+$ measured *m/z*, $[M+H]^+$ theoretical *m/z*, elemental composition (EC) (δ ppm), (RDBeq).

| No | *Rt* (min) | $[M+H]^+$ meas. m/z | $[M+H]^+$ theor. m/z | RDBeq | EC (δ ppm) | MS/MS fragments | RDBeq | Fractions derived after MCI gel purification | | | | | | | Compounds |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | *In vitro* shoot cultures | | *In vitro* tuber | | native tubers | | | |
| | | | | | | | | 40% | 60% | 40% | 60% | 20% | 40% | 60% | |
| **20** | 1.83 | 460.1969 | 460.1971 | 10.5 | $C_{24}H_{30}NO_8$ (-0.79) | 298.14 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | | + | | | | | unknown |
| **21** | 2.33 | 448.1966 | 448.1971 | 9.5 | $C_{23}H_{30}NO_8$ (-1.16) | 286.14 $[M+H-C_6H_{10}O_5]^+$ | 8.5 | | | + | | | | | unknown |
| **22** | 2.39 | 534.2336 | 534.2339 | 10.5 | $C_{27}H_{36}NO_{10}$ (-0.78) | 372.18 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | + | | + | | + | | | glycoside of **16** or **17** |
| **23** | 3.48 | 448.1975 | 448.1966 | 9.5 | $C_{23}H_{30}NO_8$ (2.11) | 286.14 $[M+H-C_6H_{10}O_5]^+$ | 8.5 | + | | + | + | | | | isomer of **21** |
| **24** | 3.83 | 474.2127 | 474.2128 | 10.5 | $C_{25}H_{32}NO_8$ (-0.30) | 312.16 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | + | + | + | | | | unknown |
| **25** | 3.83 | 460.1966 | 460.1971 | 10.5 | $C_{24}H_{30}NO_8$ (-1.26) | 298.14 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | | + | | | | | isomer of **20** |
| **26** | 4.06 | 534.1979 | 534.1975 | 11.5 | $C_{26}H_{32}NO_{11}$ (0.65) | 372.14 $[M+H-C_6H_{10}O_5]^+$ | 11 | + | + | | | | | | glycoside of **8**, **9** or **10** |
| **27** | 4.82 | 548.2133 | 548.2132 | 11.5 | $C_{27}H_{34}NO_{11}$ (-0.14) | 386.16 $[M+H-C_6H_{10}O_5]^+$ | 11 | | + | | + | | | + | colchicoside |
| **28** | 5.15 | 448.1968 | 448.1971 | 9.5 | $C_{23}H_{30}NO_8$ (-0.81) | 286.14 $[M+H-C_6H_{10}O_5]^+$ | 8.5 | | + | + | + | | | | isomer of **21** and **23** |
| **29** | 3.73 | 534.2340 | 534.2339 | 10.5 | $C_{27}H_{36}NO_{10}$ (-0.06) | 372.18 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | + | | + | | + | | | glycoside of **16** or **17** |
| **30** | 5.40 | 504.2235 | 504.2234 | 10.5 | $C_{26}H_{34}NO_9$ (0.25) | 342.17 $[M+H-C_6H_{10}O5]^+$ | 9.5 | | | + | | | | | unknown |
| **31** | 5.27 | 462.2125 | 462.2128 | 9.5 | $C_{24}H_{32}NO_8$ (-0.72) | 300.16 $[M+H-C_6H_{10}O_5]^+$ | 8.5 | | | + | + | | | | unknown |
| **32** | 4.02 | 476.2281 | 476.2284 | 10.5 | $C_{25}H_{34}NO_8$ (-0.80) | 314.14 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | | + | | | | | unknown |
| **33** | 4.61 | 476.1919 | 476.1921 | 10.5 | $C_{24}H_{30}NO_9$ (-0.41) | 314.14 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | | + | | | | | isomer of **33** |
| **34** | 4.44 | 536.2496 | 536.2946 | 9.5 | $C_{27}H_{38}NO_{10}$ (-0.01) | 374.20 $[M+H-C_6H_{10}O_5]^+$ | 8.5 | | | + | | | | | unknown |
| **35** | 3.30 | 610.2490 | 610.2500 | 10.5 | $C_{29}H_{40}NO_{13}$ (-1.55) | 448.20 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | | + | | | | | colchicodiside A or B, C |
| | | | | | | 286.14 $[M+H-C_6H_{10}O_5-C_6H_{10}O_5]^+$ | 8.5 | | | | | | | | |
| **36** | 2.74 | 490.2075 | 490.2077 | 10.5 | $C_{25}H_{32}NO_9$ (-0.38) | 328.15 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | | + | | | | | unknown |
| **37** | 3.77 | 490.2076 | 490.2077 | 10.5 | $C_{25}H_{32}NO_9$ (-0.20) | 328.15 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | | + | | | | | isomer of **36** |
| **38** | 4.23 | 472.1969 | 472.1971 | 11.5 | $C_{25}H_{30}NO_8$ (-0.51) | 310.14 $[M+H-C_6H_{10}O_5]^+$ | 11 | | | + | | | | | unknown |
| **39** | 5.34 | 536.2498 | 536.2496 | 9.5 | $C_{27}H_{38}NO_{10}$ (0.35) | 374.20 $[M+H-C_6H_{10}O_5]^+$ | 8.5 | | | + | | | | | isomer of **34** |
| **40** | 6.09 | 548.2128 | 548.2132 | 11.5 | $C_{27}H_{34}NO_{11}$ (-0.65) | 327.12 $[M+H-C_6H_{10}O_5]^+$ | 11 | | | | + | | | | unknown |
| **41** | 4.53 | 446.1813 | 446.1815 | 10.5 | $C_{23}H_{28}NO_8$ (-0.34) | 314.14 $[M+H-C_6H_{10}O_5]^+$ | 9.5 | | | | | | + | | unknown |