

1 **Cell growth kinetics and accumulation of secondary metabolite of**
2 ***Bletilla striata* Rchb.f. using cell suspension culture**

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14

15 **[Abstract]** *Bletilla striata* (Orchidaceae) is a well-recognized endangered medicinal
16 plant due to inadequate natural reproduction with high market worth. To evaluate the
17 cell growth kinetics and accumulation of secondary metabolites (SMs), the cell
18 suspension culture is proved to be a valuable approach for acquiring the high yield of
19 medicinal parts. An effective cell suspension culture for obtaining *B. striata* cell
20 growth and its SMs was *in vitro* induction of callus from *B. striata* seeds. The cell
21 growth kinetics and accumulation of SMs were analyzed using the mathematical
22 model. Results cell growth kinetic model revealed that the growth curve of *B. striata*
23 suspension cells was curved as sigmoid shape, indicating the changes of the growth
24 curve of suspension cells. Improved Murashige and Skoog cell growth medium was
25 the utmost favorable medium for *B. striata* callus formation with the highest cell
26 growth during the stationary phase of cultivation period, the cell growth acceleration
27 was started after 7 days and thereafter gradually decrease at 24 day and then reached
28 to highest at 36 day of cultivation period in both dry weight and fresh weight. The

29 coelonin concentration was peak during exponential growth stage and decreased
30 afterward at the stationary phase in the cell suspension culture. The maximum content
31 of coelonin (about 0.3323 mg/g cell dry weight) was observed on the 18th day of the
32 cultivation cycle while the dactylorhin A and militarine reached highest at 24 day, and
33 *p*-hydroxybenzyl alcohol at 39 day. This investigation also laid a foundation for
34 multi-mathematical model to better describe the accumulation variation of SMs. The
35 production of SMs had shown great specificity during cells growth and development.
36 This research provided a well-organized way to more accumulation and production of
37 SMs, on scale-up biosynthesis in *B. striata* cell suspension culture.

38 **[Key words]** *Bletilla striata*; suspension culture; growth curve; secondary
39 metabolites; kinetics; mathematical model

40

41 **1. Introduction**

42 *Bletilla striata* is a perennial herb of Orchidaceae, which is an important traditional
43 Chinese herbal medicine recorded in the pharmacopoeia of all previous dynasties. It is
44 sweet, and slightly cold with prominent effects of healing muscles and stopping
45 bleeding. Hence is ideal for treating traumatic bleeding^[1]. At present, due to the
46 limitation of the traditional artificial breeding methods, the production of *B. striata*
47 tuber and its medicinal active ingredients is difficult to meet the market demand^[2]. *B.*
48 *striata* has turn out to be an endangered class with the falling down wild plant
49 properties due to over utilization in current periods. For the sustainable progress and
50 comprehensive consumption of *B. striata*, it is essential to recognize its cell growth
51 dynamics with the accumulation of SMs. However, until now, limited approaches
52 have been developed on these perspectives. In addition, the quality and yield of
53 medicinal substances have been limited because of the deprivation of cultivated
54 variations in the course of long-period cultivation. Hence, it is needed to improve the
55 medicinal properties with upright quality, more yield, and important secondary
56 metabolites, for sustainable convention of medicinal resources.

57 With this view, the plant cell suspension culture technology can achieve artificial
58 control to provide the optimal conditions for cell growth, differentiation and
59 accumulation of SMs, therefore, we can efficiently promote cell proliferation and
60 directionally induce the synthesis and accumulation of secondary metabolites^[3]. This
61 technology has become the most promising biosynthetic method for producing
62 secondary metabolites from plant cells. Cell suspension culture systems of various
63 medicinal plants have been established at national and global level, but studies on cell
64 suspension culture and detection of secondary metabolites of *B. striata* have rarely
65 been reported^[4,5]. In the early stage, our research group has isolated, purified and
66 identified a variety of secondary metabolites from the tuber of *B. striata*^[6,7]. However,
67 it is still unknown whether these secondary metabolites are also present in *B. striata*
68 suspension culture cells.

69 On the basis of baseline research of our group, *B. striata* seeds have successfully
70 been used to induce callus and establish a rapid propagation system^[8]. Based on the
71 induction and proliferation of *B. striata* callus, in this paper, we used an induced loose
72 callus as the initial material to establish an optimized cell suspension culture system,
73 and drew the growth curve. The changes of the accumulation of four major SMs,
74 p-hydroxybenzyl alcohol, dactylorhin A, militarine and coelonin, were also detected.
75 These laid a foundation for the further development of *B. striata* cell suspension
76 culture bioreactors, as well as genetic improvement, regulation of cell proliferation,
77 SMs production, and improvement of the efficiency of producing pharmaceutical
78 important ingredients by using *B. striata*.

79

80 2. Materials and methods

81 2.1 Experimental materials

82 The loose callus induced by mature seeds of *B. striata* was used and collected from
83 the Zhengan, Guizhou, China. The callus was then inoculated on Murashige and
84 Skoog (MS) medium supplemented with 6-Benzylaminopurine (6-BA) 1.0

85 mg/L+2,4-Dichlorophenoxyacetic acid (2,4-D) 3.0 mg/L+30 g/L sucrose+7 g/L agar
86 powder and subcultured in the dark at 25°C. After 2 generations of about 30 days,
87 callus with good growth, loose texture and uniform growth was selected as the explant
88 of liquid suspension culture.

89 HPLC grade methanol for Burdick & Jackson ACS/HPLC was procured from
90 Honeywell, USA, and the standards dactylorhin A (CAS: 256459-34-4), militarine
91 (CAS: 58139-23-4), coelonin (CAS: 82344-82-9) from ChemFaces Corp. The
92 standard p-hydroxybenzyl alcohol (CAS: 623-05-2) was purchased from Chengdu
93 Ruifensi Biotechnology Corp., Ltd., China. The quality score of the reference
94 substance was more than 98%.

95

96 **2.2 Instruments**

97 BL-100A autoclave and GZX-9146MBE electric drying oven were purchased from
98 Shanghai Boxun Medical Bio Instrument Co., Ltd., China; CJHS ultra-clean
99 workbench from Tianjin Taisite Instrument Co., Ltd., China; Agilent 1260 HPLC,
100 DAD UV detector, ChemStation chromatography workstation from Agilent
101 Company, United States; BT125D analytical balance 1/100,000 from Sartorius
102 Company, Germany.

103

104 **2.3 Construction of cell suspension culture system**

105 The previously obtained loose, tender yellow callus of *B. striata* was inoculated into a
106 35 mL liquid medium (MS+6-BA 1.0 mg/L+2,4-D 3.0 mg/L+30 g/L sucrose) in a 100
107 mL flask with pH 5.8 and 1.0g per bottle (fresh weight). After inoculation, it was
108 placed in a rotary shaker, with shaking speed at 120 r•min⁻¹ at 25°C temperature under
109 dark culture condition.

110

111

112 **2.4 Determination of dry weight, fresh weight and growth curve**

113 Based on the culture conditions as stated above, the fresh and dry weight were
114 measured at every 3 days intervals after inoculation upto the 45 days taken for one
115 cycle. During the study, the flask containing the suspension cell fluid on the shaker
116 was removed, then shaken and filtered until no droplets formed. The fresh weight was
117 obtained and then dried in an oven at 50°C to a constant weight. The dry weight was
118 also measured. From each sample points, three samples were collected and measured
119 at three times. The fresh and dry weights were recorded respectively, and the growth
120 curve of the cells was plotted with the culture time as the abscissa and the fresh and
121 dry weights as the ordinate.

122

123 **2.5 Cell growth curve modeling method**

124 According to the data of fresh and dry weights of suspension cells, the growth curve
125 was plotted and analyzed by Logistic, Boltzmann and DoseResp with Origin 9.1
126 software. The best fitted model was determined by the Fitting value (R^2), and the F
127 value analyzed by ANOVA^[9]. The selected function model was used to detect the
128 acceleration rate of cell growth to analyze the proliferation of cells.

129

130 **2.6 Extraction and detection of secondary metabolites from cells**

131 **2.6.1 Preparation of test solution and chromatographic conditions**

132 According to the sampling method of 2.4, the callus of different growth stages was
133 used to detect the content of four target secondary metabolites. The sampling was
134 repeated 3 times at every 3 days intervals for each detection point. The callus was
135 filtered, dried, crushed, and passed through the sieve ($\Phi 200 \times 50$ mm). Approximately
136 0.20 g of *B. striata* cells were accurately weighed, mixed with 100 ml of 70%
137 methanol water for 2 hours into the condensation reflux extraction, and centrifuged to
138 obtain an extract liquid. The recover extract liquid was dried under reduced pressure,

139 dissolved in an appropriate amount of 70% methanol water, and then transferred to a
140 5 mL volumetric flask for use as a test solution. HPLC detection conditions were as
141 follows; Column: Dubhe C₁₈ (250 mm × 4.6 mm, 5 μm); mobile stage: methanol (A),
142 ultrapure water (B); flow rate: 0.8 mL / min; column temperature: 25 °C; detection
143 wavelength: 225 nm^[10]; injection volume: 20 μL. The changes of volume fraction
144 during the gradient elution process is as follows: mobile stage methanol (A) -
145 ultrapure water (B), gradient elution (0-10 min, 20%-25% A; 10-25 min, 25 %-50%
146 A; 25-35 min, 50%-50% A; 35-50 min, 50%-100% A; 50-60 min, 100%-100% A).

147 **2.6.2 Study on the linear relationship of the standard curve**

148 A suitable concentration of p-hydroxybenzyl alcohol, dactylorhin A, militarine,
149 coelonin were prepared for standard solution, and diluted with methanol to prepare a
150 series of mass concentrations (0.25, 0.2, 0.15, 0.1, 0.05, 0.025 mg/mL for
151 p-hydroxybenzyl alcohol, 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 mg/mL for militarine, 0.1,
152 0.05, 0.025, 0.01, 0.005, and 0.0025 mg/mL for coelonin). The detection was done
153 under the standard chromatographic conditions as described in the section **2.6.1**.

154 **2.6.3 Validation of HPLC method**

155 **2.6.3.1 Precision experiment**

156 The standard solutions as described in the section **2.6.2** were precisely taken, and 20
157 μL set for injection volume. The sample was detected under the chromatographic
158 conditions of **2.6.1** and repeated for 6 times.

159 **2.6.3.2 Stability experiment**

160 Sample solution (as described in **2.6.1**) was injected at 0, 3, 6, 9 and 12 hours after
161 preparation. Peak areas of p-hydroxybenzyl alcohol, dactylorhin A, militarine and
162 coelonin were recorded, and the injection volume was 20 μL.

163 **2.6.3.3 Repeatability of experiment**

164 Take 5 *B. striata* cell clusters, each of which was weighed 0.20 g. The samples were
165 prepared according to the method under **2.6.1**. All samples were injected under the

166 same chromatographic conditions. The injection volume was 20 μ L. The peak areas
167 were recorded and calculated the the mass fractions of p-hydroxybenzyl alcohol,
168 dactylorhin A, militarine and coelonin. The relative standard deviation (RSD) was
169 calculated in according to the formula.

170 $RSD(\%) = STDEV/AVERAGE*100$

171

$$SD = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$

172 **2.6.3.4 Sample recovery analysis**

173 Nine *B. striata* suspension cultured cell clusters with known secondary metabolites
174 were accurately weighed, each of which was weighed 0.02 g, and precisely added to
175 three reference solutions with low, medium and high mass concentrations (80%,
176 100%, 120% of the original sample, respectively). There were 3 reference substances
177 in each mass concentration. The recovery rate and RSD of each components were
178 calculated.

179

180 **2.7 Modeling method for cumulative curve of secondary metabolites**

181 The data of the cumulative number of secondary metabolites of *B. striata* suspension
182 cells were fitted by a variety of functional models under the “Nonlinear Curve Fit” in
183 Origin 9.1 software and determined the best fitted model by the fitness value (R^2).

184

185 **3 Results**

186 **3.1 Growth curve of suspension cultured cells**

187 The growth curve of suspension culture cells was plotted with the fresh and dry
188 weight as the indicators. As shown in Figure 1, it was found that the growth curves of
189 the two different growth indicators was basically the same and both were "sigmoid"

190 type.

191

192 **3.2 Growth curve and kinetic characteristics**

193 **3.2.1 Functional model of growth curve**

194 The mathematical models were fitted and analyzed for the change of fresh and dry
195 weight of suspension culture cells. The results are shown in Table 1.

196 As shown in Table 1, the coefficient of determination of the logistic, Boltzmann
197 and DoseResp fitting equations for the fresh weight and days were 0.9817, 0.9231,
198 and 0.9780, respectively. The coefficient of determination of the three fitting
199 equations for dry weight and days were 0.9761, 0.8878 and 0.9453, respectively.
200 According to the fitting results, the Logistic model was more suitable to describe the
201 growth of biomass in suspension cells of *B. striata*. After a further significance test,
202 the F values of Logistic equation (721.56294 and 613.94951) were also higher than
203 Boltzmann equation and Dose Resp equation, indicating that the curve of Logistic
204 equation is more consistent with the experimental data. Logistic equation was further
205 used to plot the curve of the fresh and dry weight growth of suspension cultured cells
206 (Fig. 2).

207 The results showed that the whole culture cycle could be divided into six stages: lag
208 stage, exponential stage, linear stage, deceleration stage, stationary stage and
209 recession stage. Among them, the lag stage was found on 0 to 6 day. During this
210 period, the fresh and dry weight of suspension culture cells were changed slowly,
211 indicating that the cells gradually adapted to the environment. During the exponential
212 stage (between 6-12 day), the cell growth rate was gradually increased and reached to
213 the maximum. The growth rate of callus was gradually stable during the linear stage
214 of 12-24 day, and the change of fresh and dry weight was linearly correlated with
215 time. The deceleration stage was lasted from 24 to 36 day, during which the cell
216 growth rate gradually decreased. At 36 day, the fresh and dry weight of cells were
217 reached the maximum. While they did not change significantly during the stationary

218 stage of 36-39 day while these two weights were beginning to decrease after 39 day.

219 **3.2.2 Cell growth curve function model analysis**

220 The Logistic function was presented the cumulative amount of cell growth. In order to
221 better understand the changes in suspension culture cell growth, we performed
222 first-order derivative (cell growth rate) and second-order derivative (cell growth
223 acceleration) of the simulated Logistic function and plotted the results of the
224 derivation, as shown in Figure 3. It is reflected from the figure that the growth rate of
225 the cells was increased at initially. When it reached to its peak, it was affected to
226 decrease gradually by various factors and the growth rate. The overall trend was first
227 increased and then decreased. The extremums of the two curves showed that the
228 growth acceleration of suspension cultured cells was reached the maximum on the 7
229 day and the cell growth rate reached the maximum on 13 to 14 day.

230

231 **3.3 Measurement of secondary metabolites accumulation in suspension cultured**
232 **cells**

233 **2.3.1 Investigation of specificity**

234 The chromatograms samples were tested with reference substances, filtrate of
235 suspension culture cells and blank solution are shown in Fig. 4. The results shown that
236 the absorption peaks of the sample was tested at the same conditions as the reference
237 substance, and the blank solution for no interference. Moreover, it showed that there
238 was no obvious number of secondary metabolites left in the cell culture medium, so
239 the samples were accurately reflected the accumulative amount of secondary
240 metabolites in suspension culture cells.

241 **2.3.2 Investigation of linear relations**

242 The standard curves were drawn with the mass concentration of p-hydroxybenzyl
243 alcohol, dactylorhin A, militarine and coelonin as the abscissa (X) and the four
244 secondary metabolites corresponding peak area as the ordinate (Y₁₋₄). The regression

245 equations were $Y_1 = 91387X - 169.99$ ($R^2 = 0.9994$), $Y_2 = 36075X - 712.50$ ($R^2 = 0.9996$),
246 $Y_3 = 24341X - 224.88$ ($R^2 = 0.9993$) and $Y_4 = 69896X - 142.88$ ($R^2 = 0.9994$).

247 **2.3.3 Validation of the methodology of HPLC**

248 The results of precision test showed that RSD of peak areas of p-hydroxybenzyl
249 alcohol, dactylorhin A, militarine and coelonin were 0.83%, 0.40%, 1.36%, 1.24%,
250 respectively while RSD of retention time were 0.09%, 0.08%, 0.07%, 0.16%,
251 respectively. The results of stability test showed that the RSD of peak areas of the
252 four components were 0.71%, 1.73%, 1.86% and 2.80% respectively, while RSD of
253 retention time were 0.86%, 0.50%, 0.76% and 1.61% respectively, indicating the
254 solution had good stability within 12 hours. The results of repeatability experiment
255 showed that the RSD of the four components were 2.19%, 2.54%, 0.78% and 2.00%,
256 respectively, which indicated good repeatability. The recovery experiment of four
257 secondary metabolites results were 1.59%, 1.85%, 1.24% and 1.98% showed that the
258 method had good accuracy.

259 **3.4 Kinetic characteristics of secondary metabolites accumulation in suspension
260 system**

261 The accumulation of the four secondary metabolites in cells of different growth stages
262 were calculated, and plotted as curves. The mathematical models were used to
263 describe the changes of the accumulation of the four chemicals, as shown in Table 2.
264 The results showed that the changes of secondary metabolites in suspension cultured
265 cells were more complicated than that of cell growth. A single mathematical model
266 could not well describe the measured data. Therefore, for the three secondary
267 metabolites (dactylorhin A, militarine, coelonin), the multi-mathematical model and
268 piecewise function were selected to describe the change of their cumulative amount,
269 as shown in Figure 5.

270 According to the figure, the accumulation of p-hydroxybenzyl alcohol was similar
271 to cell growth, showing a gradual upward trend. The curve went smoothly, and the
272 cumulant increased slowly at 24 day, and the accumulation reached the maximum at

273 39 day. There was a distinct maximum value in the cumulant curves of dactylorhin A,
274 militarine and coelonin. The cumulative amount of dactylorhin A and militarine both
275 reached the maximum at 24 day, and the cumulative amount of coelonin reached the
276 maximum at 18 day.

277

278 **4 Discussion and conclusion**

279 Compared with plant cultivation, cell culture has the advantages of short growth
280 cycle, easy separation of secondary metabolites and easy control of influencing
281 conditions. Plant cell culture makes it easier to obtain specific natural products from
282 medicinal plants by specifically inducing the synthesis of specific secondary
283 metabolites. Therefore, using mathematical models to analyze cell growth and
284 secondary metabolite synthesis and accumulation is important for revealing the
285 synthesis mechanism of natural products such as secondary metabolic components,
286 improving the yield of secondary metabolites and enhancing the medicinal value of
287 medicinal plants^[11]. In recent years, with the deepening of relevant researches, the
288 medicinal value of various secondary metabolites in *B. striata* has been confirmed by
289 scientific research. For example, p-hydroxybenzyl alcohol can increase the expression
290 of genes encoding antioxidant proteins after focal cerebral ischemia, which can avoid
291 oxidative stress and further damage on brain neurons^[12]. The dactylorhin A and
292 militarine can significantly improve memory impairment in mice which is caused by
293 chemicals such as scopolamine, cycloheximide and alcohol^[13] while
294 2,7-dihydroxy-4-methoxy-9,10-dihydrophenanthrene (coelonin) has certain antiviral
295 activity as a kind of dihydrophenanthrene compound. It is more meaningful to use
296 plant cell liquid culture technology to regulate the synthesis of various secondary
297 metabolites^[14].

298 In this study, we simulated the growth of *B. striata* suspension culture cells with a
299 variety of mathematical function models, and properly simplified the complex
300 environment in which a group of cells were grown together with the mathematical

301 model of growth kinetics. From the results of function fitting, all the mathematical
302 models could accurately describe the growth of cells at different growth stages and
303 the changes of secondary metabolites accumulation. The growth of suspension culture
304 cells are divided into six stages: lag stage, exponential stage, linear stage, deceleration
305 stage, stationary stage and recession stage. Between 13 and 14 day, the cell growth
306 rate was reached its maximum. After 39 day, the cell growth did not show a
307 significant decline trend in the functional model, but according to the actual data and
308 observation of the state of the culture cells, we found that the culture cells showed
309 obvious browning, indicating the growth entered the recession stage. These
310 phenomena were well presented in the functional model.

311 However, the changes of accumulation of secondary metabolites in the suspension
312 culture cells of *B. striata* were complicated and diversified. The change in the
313 cumulative amount of p-hydroxybenzyl alcohol basically followed the change in the
314 growth of *B. striata* suspension culture cells, while the content changes of dactylorhin
315 A, militarine and coelonin were not consistent with the growth of cells. By comparing
316 the growth curve with the change curve of the accumulation of secondary metabolites,
317 when the cell growth reached the end of the linear growth stage (12-24 day), the
318 accumulation of secondary metabolites generally declined. At this stage, due to the
319 reduction of nutrients in liquid medium, the environmental inhibition began to be
320 greater than the cell growth, and secondary metabolism, as a non-essential substance
321 of plant life, would gradually decrease. Dactylorhin A and militarine, as a glycoside
322 compound, are both secondary metabolites and energy storage substances that
323 actively decompose to maintain life in adversity, which may result in a decrease in
324 secondary metabolites in cells^[15]. Fermentation kinetics studies on microorganisms
325 have shown that p-hydroxybenzyl alcohol itself can act as an inducer to induce fungi
326 to increase extracellular polysaccharide production. In this study, we found that the
327 curve of p-hydroxybenzyl alcohol accumulation gradually became smooth after 24
328 day, while dactylorhin A and militarine reached maximum values at the 24th day. The
329 effects of p-hydroxybenzyl alcohol on the key enzymes of polysaccharide synthesis in

330 plant cells and some possible factors of synthesizing promotion need to be further
331 studied. It is well known that the production of secondary metabolites by plants are
332 the result of their adaptation to the ecological environment during the long-term
333 evolution. The synthesis of these secondary metabolites is closely related to the
334 growth state of plants and environmental conditions. Different types of secondary
335 metabolites have different biosynthetic pathways in the different stages of plant
336 growth.

337 Further studies are needed to control the metabolic pathways, key enzymes and key
338 regulatory genes; so that we can improve the efficiency of suspension culture of *B.*
339 *striata* cells and achieve efficiently directive induction of secondary metabolites by
340 means of genetic engineering and metabolic engineering^[16]. It also lays a theoretical
341 and practical foundation for the follow-up researches on the growth, mutant induction,
342 and secondary metabolite synthesis and bioreactor construction of *B. striata*.

343

344 **Acknowledgments**

345 This research was financially supported by the National Natural Science Foundation
346 of China (31560079, 31560102), the Scientific Project of Guizhou Province
347 (QKH-ZY[2013]3002, QKH-LH[2014]7549, [2017]5733-001), Talents Promotion
348 Project of Zunyi Medical University(JC2018-2-5(1)), the PhD Science Foundation of
349 Zunyi Medical University (F-809), and the Talent Growth Project of Guizhou
350 Education Department (KY[2017]194).

351

352 **Interest statement**

353 The authors declare that they have no conflict of interest.

354

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415

416 **Figure legends:**

417 Figure 1 Growth curve of fresh and dry weights of suspension cells in culturing days.

418 Figure 2 Growth kinetics curves of fresh and dry weights of suspension cells.

419 Figure 3 Function graphs of the first derivative and the second derivative of Logistic
420 model.

421 Figure 4 HPLC chromatograms of various constituents. a. Test samples. b. Reference
422 substances. c. the filtrate of suspension cultured cells of *Bletilla striata*. d. Blank
423 solution (70% methanol). 1. p-hydroxybenzyl alcohol. 2. dactylorhin A. 3. militarine.
424 4. coelonin.

425 Figure 5 Content change and fitting curves of four secondary metabolites. a.
426 p-hydroxybenzyl alcohol. b. dactylorhin A. c. militarine. d. coelonin.

428 **Tables and captions:**

429 Table 1 Mathematical modeling results of the growth curve of fresh weight and dry
430 weight of suspension culture cells.

431 Table 2 Mathematical model fitting results of four secondary metabolite content.

432

433 **Supporting information**

434 Figure S1. Morphology of callus in different growth periods. (.docx)

435 Figure S2. Growth states of suspension culture cell. (.docx)

436 Figure S3. Standard curve of four secondary metabolites. (.docx)

437 Table S4. Examination results of precision. (.docx)

438 Table S5. Examination results of stability. (.docx)

439 Table S6. Examination results of reproducibility. (.docx)

440 Table S7. Results of recovery tests for tested chemicals. (.docx)

441 Table S8. Dry and fresh weight growth of suspension cultured cells in 45-day culture
442 period. (.xlsx)

443 Table S9. Mean measurement of secondary metabolites. (.xlsx)

444

445 **Table 1**

Trait	Mathematical model	Parameter				
		A1	A2	x0	p/dx/ LOGx0	R ² /F
fresh weight	Logistic $f(x) = A2 + (A1-A2)/(1+ (x/x0)^p)$	1.05 40	4.729 0	17.08 45	p=3.0	0.9817 721.56294
	Boltzmann $f(x) = A2 + (A1-A2)/(1 + \exp((x-x0)/dx))$	1.05 40	4.729 0	17.08 45	dx=2.25	0.9231 168.85936
	DoseResp $f(x) = A1 + (A2-A1)/(1 + 10^{(LOGx0-x)*p})$	1.05 40	4.729 0	-	p=0.11 LOGx0=1 7.08	0.9780 596.33032
dry weight	Logistic $f(x) = A2 + (A1-A2)/(1+ (x/x0)^p)$	0.10 32	0.429 2	14.07 85	p=3.0	0.9761 613.94951
	Boltzmann $f(x) = A2 + (A1-A2)/(1 + \exp((x-x0)/dx))$	0.10 32	0.429 2	14.07 85	dx=2.25	0.8878 128.45275
	DoseResp $f(x) = A1 + (A2-A1)/(1 + 10^{(LOGx0-x)*p})$	0.10 32	0.429 2	-	p=0.11 LOGx0=1 4.08	0.9453 266.3732

446

447 **Table 2**

Secondary metabolite	Mathematical model	Functional equation	R ²
p-hydroxybenzyl alcohol	SGompertz	$f(x) = 1.0489 * \exp(-\exp(-0.0886 * (x - 3.1379)))$	0.9121
dactylorhin A	Exponential (if $x \leq 24$)	$f(x) = 5.7649 + 0.9840 * \exp(0.1084 * x)$	0.9015
	ExpDec1 (if $x \geq 24$)	$f(x) = 365.0176 * \exp(-x/7.1996) + 6.0012$	0.9568
militarine	GaussAmp (if $x \leq 24$)	$f(x) = 2.2007 + 4.1171 * \exp(-0.5 * ((x - 24) / 6.9654)^2)$	0.8443
	Bradley (if $x \geq 24$)	$f(x) = -21.9151 * \ln(0.2391 * \ln(x))$	0.9715
coelonin	BiDoseResp (if $x \leq 18$)	$f(x) = 0.1658 + 0.0833 / (1 + \text{pow}(10(2.5492 - x) * 0.2778)) + 0.0833 / (1 + \text{pow}(10(13.2375 - x) * 0.2778))$	0.9372
	DoseResp (if $x \geq 18$)	$f(x) = 0.201 + 0.1313 / (1 + 10^{((26.6223 - x) * -0.1852)})$	0.9038

448

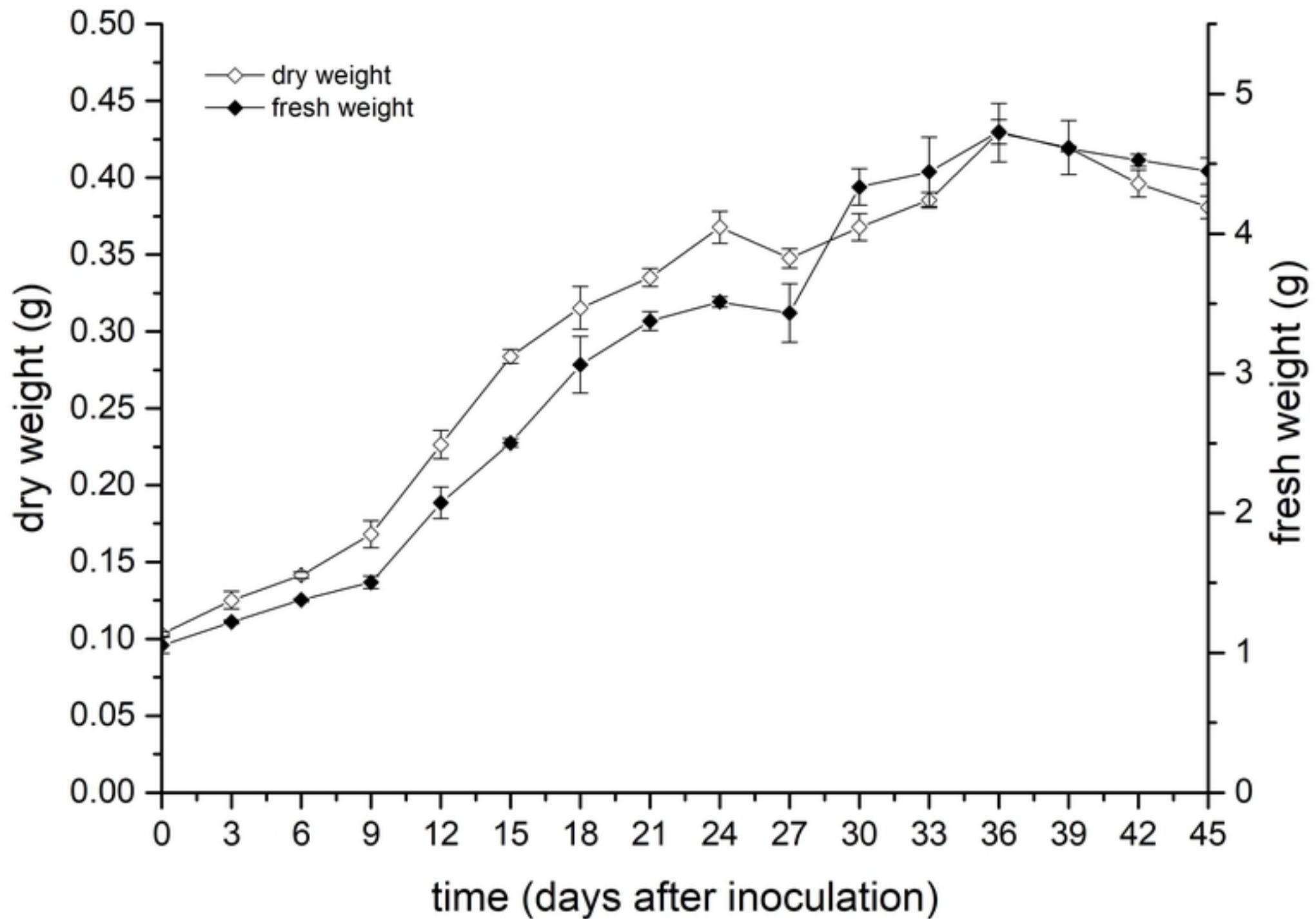


Figure 1

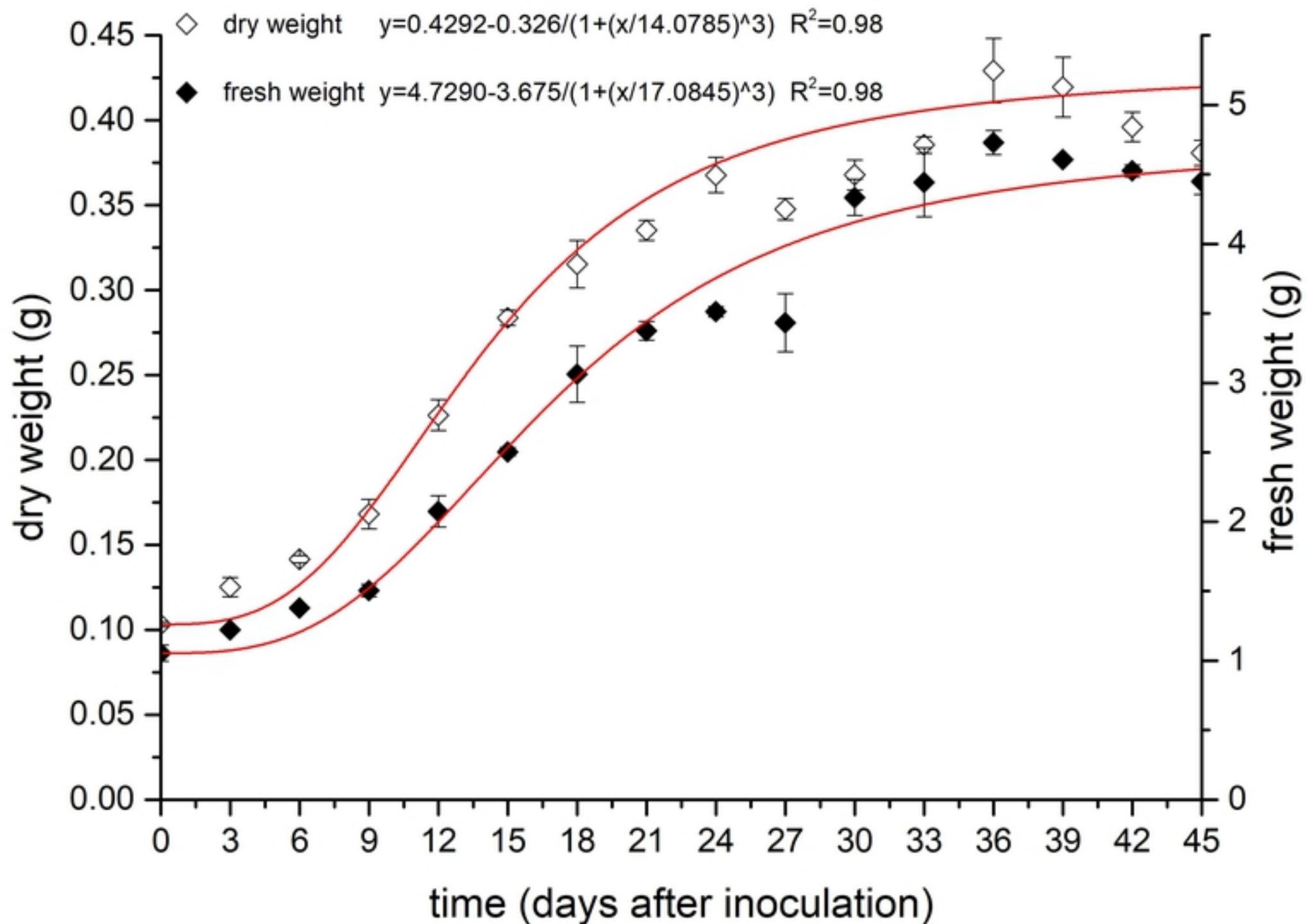


Figure 2

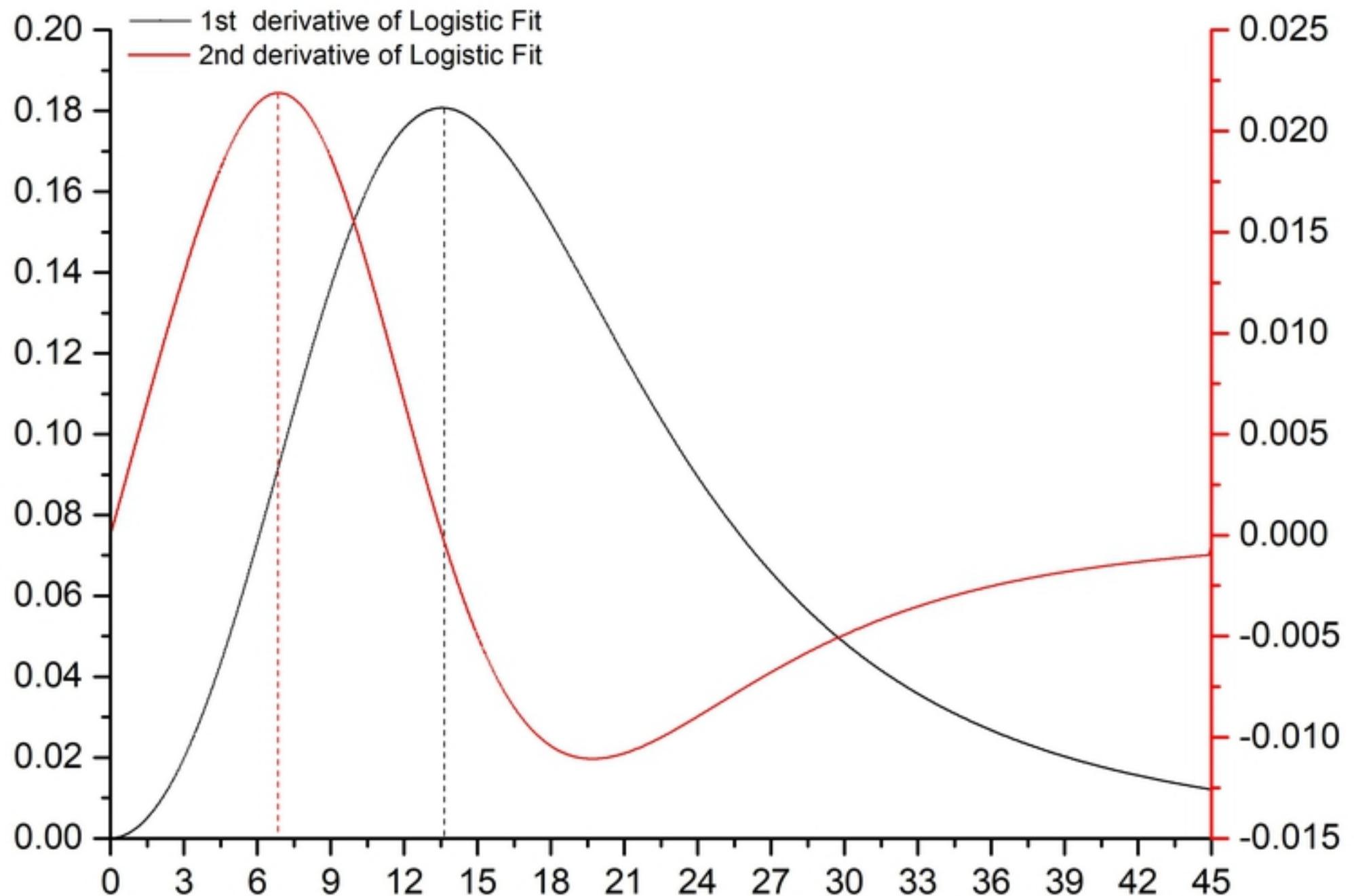


Figure 3

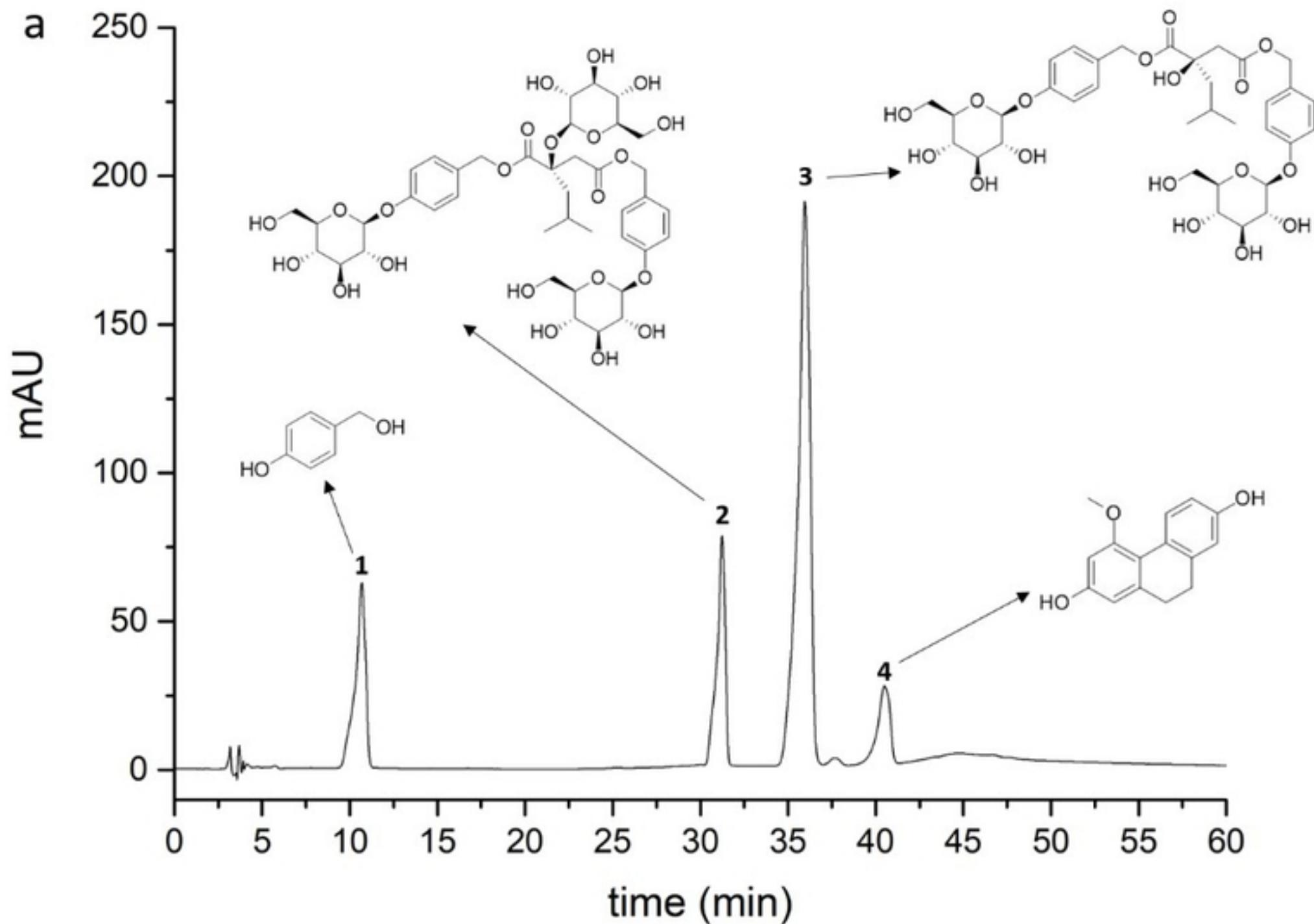


Figure 4

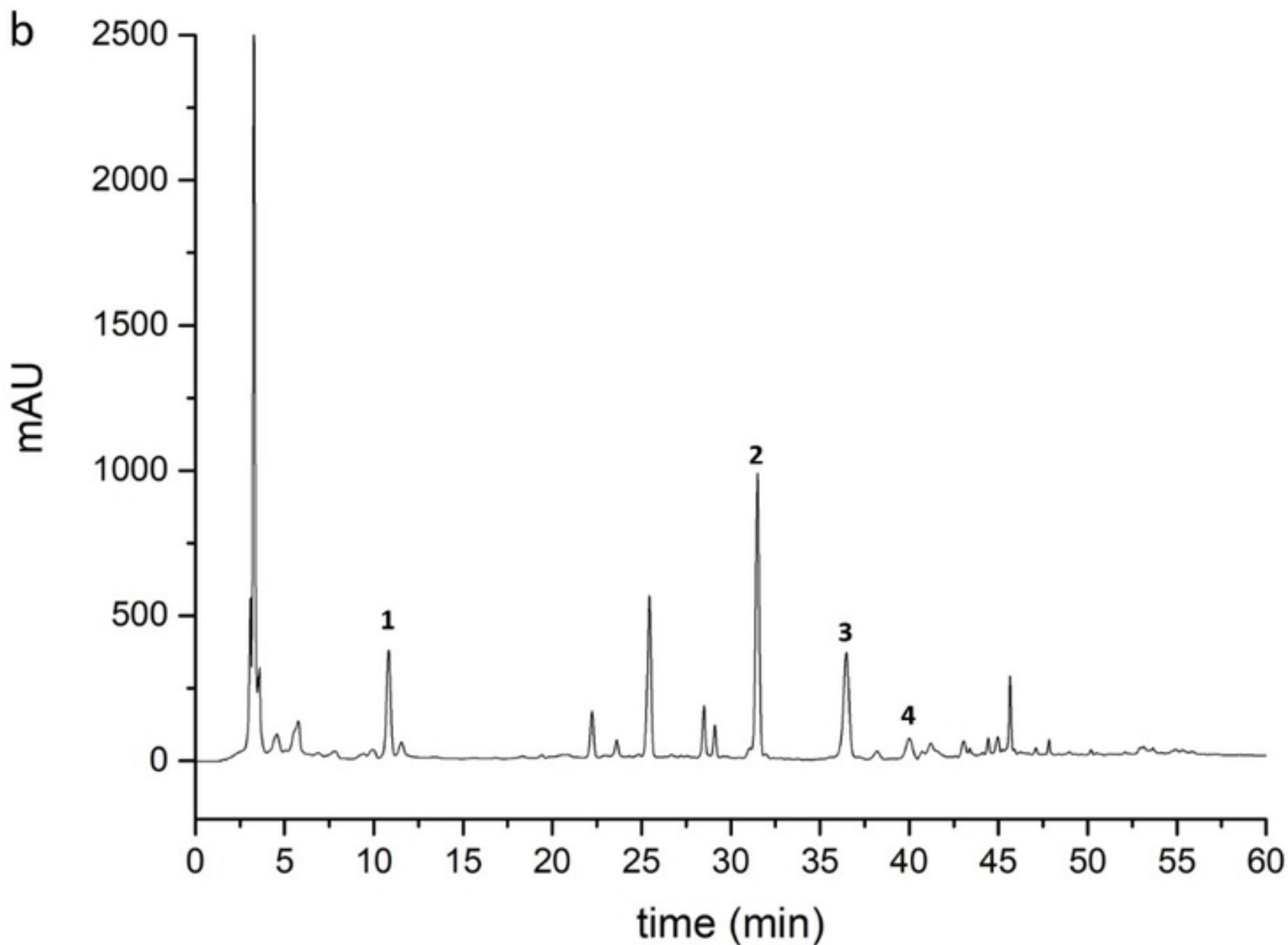


Figure 5

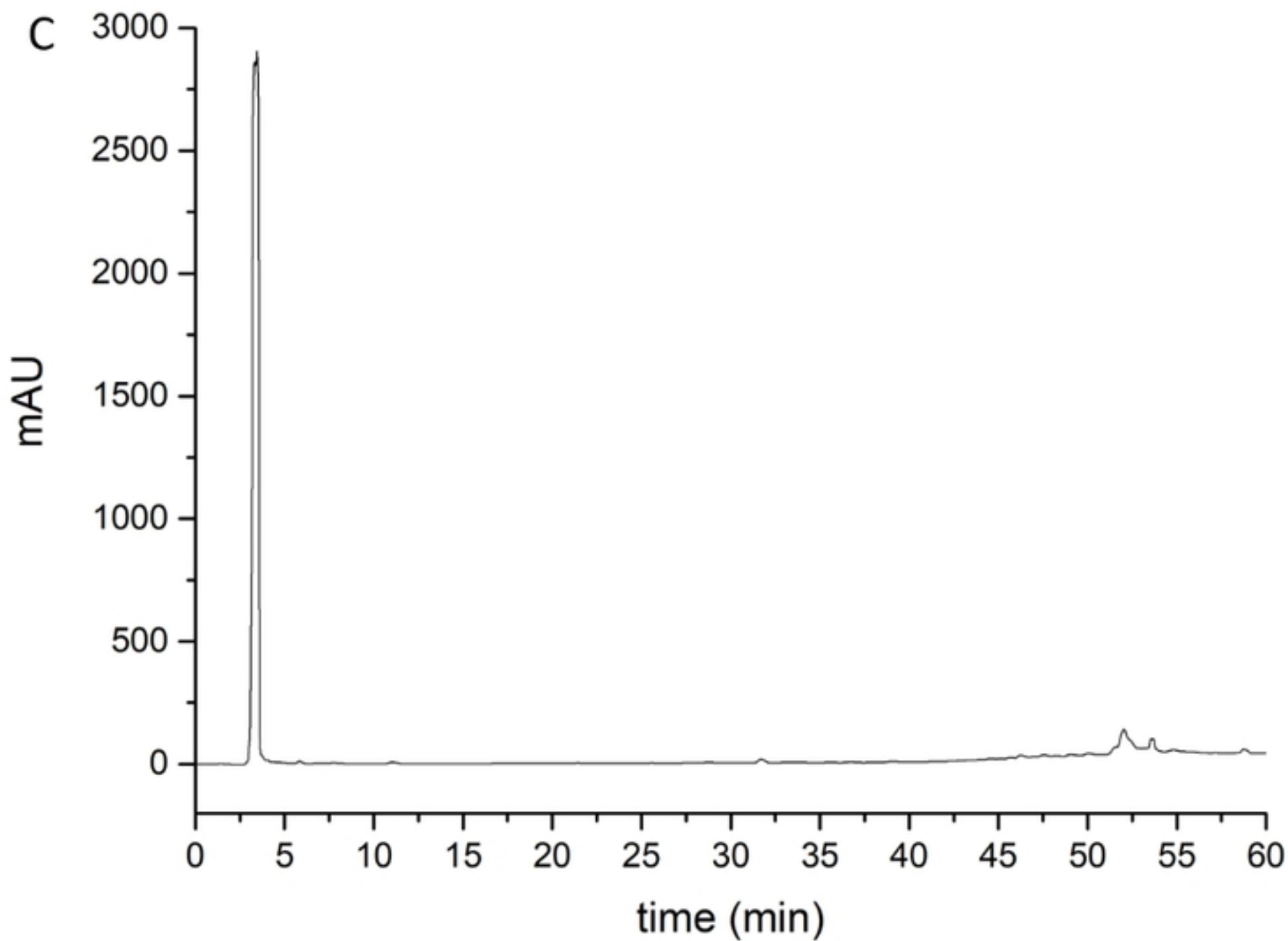


Figure 6

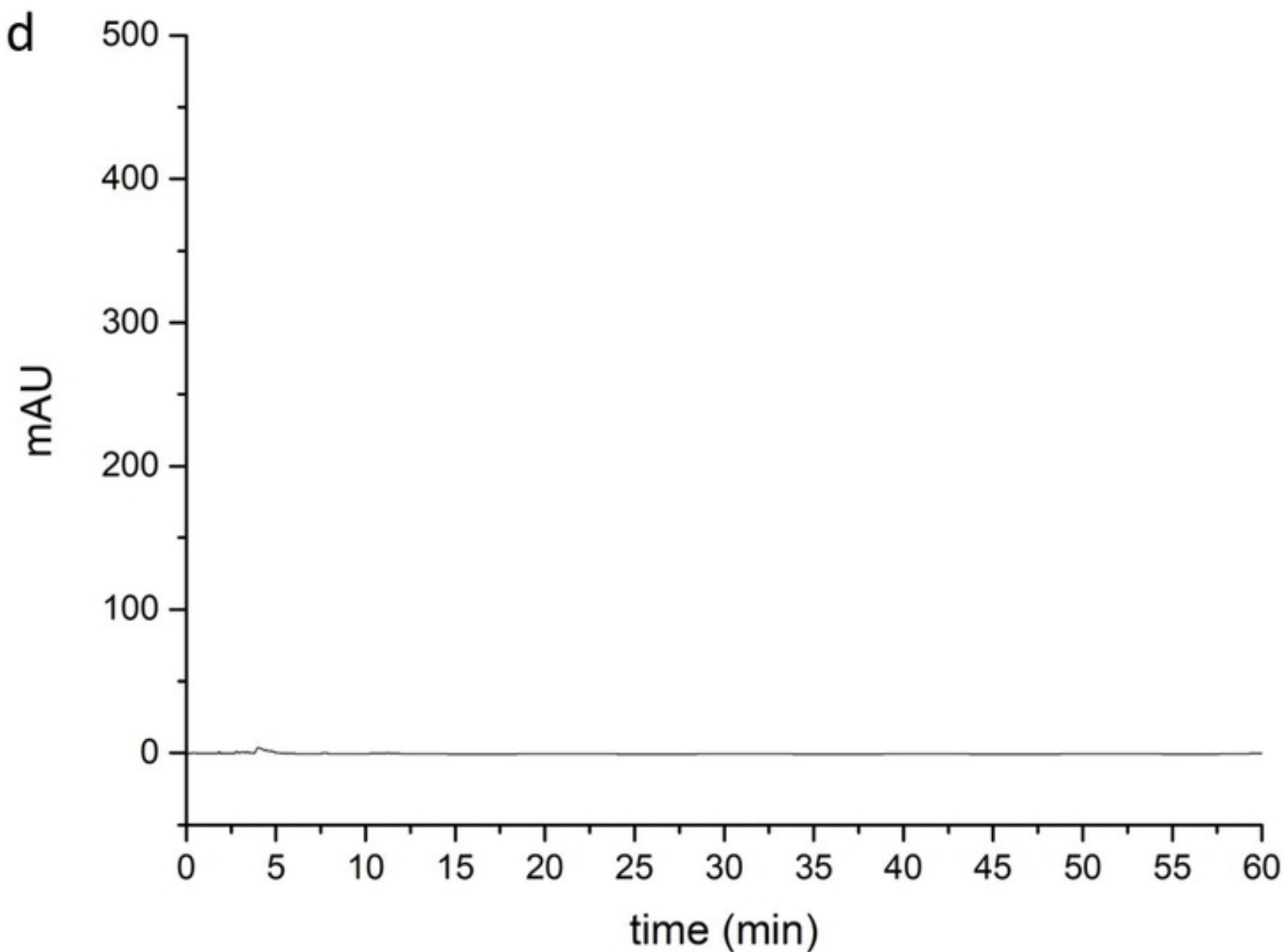


Figure 7

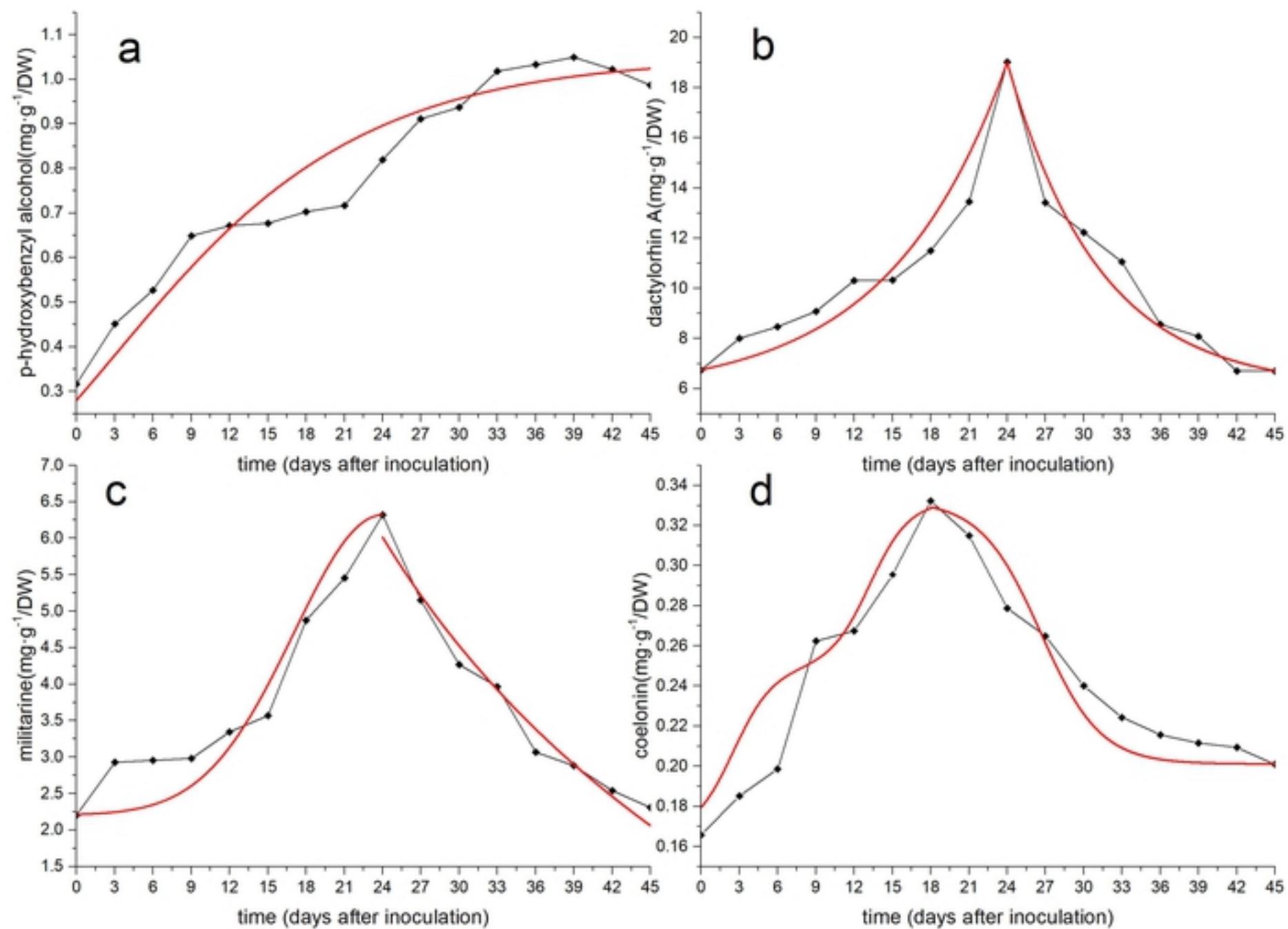


Figure 8