

Plant Biotechnology

Advance Online Publication by J-STAGE

<https://doi.org/10.5511/plantbiotechnology.24.0717a>

Received February 19, 2024; Accepted July 17, 2024

Published Online October 12, 2024

Copyright c 2024 Japanese Society for Plant Biotechnology. This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 License (CC BY, <https://creativecommons.org/licenses/by/4.0/>).

Original Paper

Analysis and characteristics of coronaridine, an alkaloid found in *Catharanthus roseus*.

Hiroaki Kisaka, Sachise Karakawa, Tetsuya Miwa, Hiroto Hirano, Takashi Onuki, Mayu Iyo

Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co. Inc. 1-1, Suzuki-Cho,
Kawasaki-ku, Kawasaki-Shi 210-8681, Japan

A. Corresponding author: Hiroaki Kisaka

Address: 1-1 Suzuki-Cho, Kawasaki-ku, Kawasaki-Shi 210-8681, Japan

Phone: 070-4300-8008

E-mail: hiroaki.kisaka.f2q@asv.ajinomoto.com

B. Running title: Characterization of coronaridine in *Catharanthus roseus*

C. Abbreviations:

CD, circular dichroism spectrum; GC–MS, gas chromatography–mass spectrometry; LC–MS, liquid chromatography–mass spectrometry; RT, retention time

Abstract

Coronaridine, a monoterpenoid indole alkaloid, is present in *Tabernanthe iboga* and the related species *Tabernaemontana divaricata*. Recent exhaustive analysis revealed its presence in *Catharanthus roseus*, though specific details remain unknown. We conducted a detailed analysis of coronaridine in *C. roseus*, detecting it in seedlings post-germination up to 8 weeks after sowing, with peak abundance at 3–4 weeks. Gradual decrease occurred from the flowering stage, and it was absent during seed formation. The accumulation varied dramatically with the plant's growth phase. LC–MS/MS analysis confirmed (–) coronaridine, consistent with *T. iboga*. Additionally, cultivating at 35 °C increased coronaridine accumulation over 10-fold. These findings hold potential for enhancing the stable production of iboga alkaloids for pharmaceutical use.

Key words:

alkaloid, *Catharanthus roseus*, coronaridine, high temperature, LC-MS/MS

Introduction

Various species within the *Apocynaceae* family, such as *Tabernanthe iboga*, *Voacanga africana*, and numerous *Tabernaemontana* species, are recognized for producing iboga-type alkaloids. Ibogaine, an iboga-type alkaloid, has shown promise as a medicinal drug. Several studies, both in animals and in humans, report long-term drug abstinence from various substances, including opioids, alcohol, and psychostimulants, and sustained reductions in depressive symptoms after ibogaine administration (Mash et al. 2000; Schenberg et al. 2014; Santos et al. 2017; Brown and Alper, 2018; Mash, 2018; Mash et al. 2018; Wasko et al. 2018). *T. iboga* is a plant native to Africa and is a limited resource. This fact making it difficult to ensure a stable supply as a pharmaceutical ingredient.

Farrow et al. (2018) examined iboga alkaloid metabolism in *Tabernanthe iboga*, revealing coronaridine as precursors. Their study reported two enzymes, cytochrome P450 (I10H) and *O*-methyltransferase (N10OMT), essential for iboga alkaloid biosynthesis from coronaridine. Coronaridine (Figure 1), also known as 18-carbomethoxyibogamine, is an alkaloid found in *Tabernanthe iboga* and related species, including *Tabernaemontana divaricata* (Singh et al. 2011).

Catharanthus roseus, also a member of the *Apocynaceae* family, is a widely cultivated flowering plant known for generating approximately 130 different alkaloids. Among these, vincristine and vinblastine are prominent compounds used to treat leukemia and Hodgkin's lymphoma (Dhayanithy et al. 2019). *C. roseus* also contains relatively large amounts of alkaloids such as catharanthine, vindoline, and ajmalicine (Figure 1), and catharanthine is classified as an iboga-type alkaloid. Recent gas chromatography–mass spectrometry (GC–MS) analysis has identified the presence of coronaridine in *C. roseus* (Rani et al. 2021; Bansal et al. 2023). If *C. roseus* contains large amounts of coronaridine, it may be possible to semisynthetically produce iboga alkaloids using that coronaridine. Therefore, we focused on and characterized the coronaridine found in *C. roseus*.

Materials and Methods

Cultivation and sampling of *C. roseus* plants

The seeds of *C. roseus* used were Pacifica XP cv Really Red (Takii Seed Co., Ltd). Two seeds were sown in watered Jiffy Seven (Sakata Seed Corporation). Cultivation occurred at 25 °C temperature, 60 % humidity, and 100–150 $\mu\text{mol m}^{-2}\text{S}^{-1}$ light amount. After one week, one fully germinated plant was retained, and another was removed. Following another week of cultivation, plants were transferred to 6-cm pots with

supplemented culture soil. Otsuka Liquid Fertilizer (OAT Agrio Co., Ltd.) at a 2,000-fold dilution was used for weekly plant fertilization.

Plants cultivated at different temperatures were transplanted into soil and grown at either 25 °C or 35 °C for 3 weeks. Three plants with uniform growth were selected, and 0.2 g of leaves were sampled from the second and third leaves from the growth point. To confirm the difference in the amount of coronaridine accumulated in young and old leaves, we used three plants grown at 25 °C for 8 weeks after sowing. 0.2 g of the 2nd to 3rd leaf from the growth point or the 4th to 5th leaf from the base was sampled. The plants sown weekly were transplanted to 35 °C one week after sowing, and one week later, they were planted in soil and continued cultivation at 35 °C. Three independent plants were prepared for each week-old plot, and 0.2 g of each plant was sampled using the 2nd to 4th leaves from the growth point. A 3-mm diameter zirconia bead was placed in a 2-mL microtube (Eppendorf Co., Ltd), and 0.2 g of measured leaf material was added. The samples were frozen using liquid nitrogen, and stored at -80 °C until all samples were collected.

Alkaloid extraction

A frozen sample was crushed with MM400 (Retsch), and 1 mL of methanol was added to a 2-mL microtube. Using a microtube mixer (Tokyo Rikakikai), the samples were mixed at 2,000 rpm and 28 °C for 2 h. Then, centrifugation was performed at 25 °C at 14,000 × g for 20 min, and the supernatant was filtered using an Ultra-free-MC 0.22-μm filter unit (Millipore). The filtrate was used for analysis.

Solid-phase extraction conditions

A mobile phase A solution was prepared using 10 mM ammonium acetate, 0.1 % acetic acid, and water. The methanol extract of the sample (200 μL) was mixed with 3800 μL of mobile phase A. Oasis HLB 3 mL (60 mg) Extraction cartridges (Waters) served as the solid-phase extraction column. Column conditioning included 2 mL of methanol, 1 mL of water, and 2 mL of mobile phase A. Loading the column with 4 mL of the sample was followed by a wash with 3 mL of 70 % methanol in water. Elution utilized 1.5 mL of 100 % methanol, and the eluate was dried under reduced pressure at 45 °C for 1 h. After adding and dissolving 60 μL of methanol, 40 μL of water was added and mixed to prepare the analysis sample.

LC– MS and LC– MS/MS measurements

We were kindly provided with standard coronaridine by professor Hirosawa of Hoshi University. They conducted research on the isolation and usefulness of monoterpenoid indole alkaloid from *Tabernaemontana divaricata*, and coronaridine was one of these alkaloids (Hirasawa et al. 2021). Coronaridine was analyzed by LC–MS using a Nexera X2 HPLC system from Shimadzu (Kyoto, Japan) and Q-Exactive™ puls from Thermo Fisher Scientific (Sunnyvale, CA, USA). A Waters Acquity UPLC® BEH C18 (2.1 × 100 mm, 1.7 μm) column was used for analysis. Mobile phase A: 10 mM ammonium acetate/0.1 % acetic acid/water; mobile phase B: methanol; flow rate: 1 mL/min; column temperature: 40°C; and gradient conditions: 0–30 min from 60 % to 95 % mobile phase B, 30–40 min maintained at 95 %, 40–40.1 min from 95 % to 60 %, and 40.1–50 min at 60 % for reequilibration. The injection volume was 10 μL. PDA detection was performed at 190–400 nm.

In the MS system, full mass scan measurement and PRM measurement (MS/MS of target analyte) were performed. The full mass scan parameters were set as follows: polarity, positive; scan range, 100–1000 *m/z*; resolution, 70000; AGC target, 3×10^6 ; and maximum IT, 200 ms. The PRM parameters were set as follows: resolution, 17,500; default charge state, 1; AGC target, 2×10^5 ; maximum IT, 100 ms; isolation window, 4.0 *m/z*. The collision energy (CE) was set at 30, 50, and 60 V.

LC-CD measurement

For LC-CD analysis, a JascoX-LC system (JASCO Corporation) and CD-2095 Plus detector (JASCO Corporation) were used. The column was Intertsil ODS-3 (4.6 × 250 nm, 5 μm) (GL Science, Tokyo, Japan), the column temperature was 40°C, mobile phase A was 10 mM ammonium acetate/0.1 % acetic acid/water, and mobile phase B was methanol. Flow rate: 1 mL min⁻¹; injection volume: 10 μL. The gradient conditions were as follows: 0–30 min from 60 % to 95 % mobile phase B, 30–40 min maintained at 95 %, 40–40.1 min from 95 % to 60 %, and 40.1–50 min at 60 % for reequilibration. PDA detection was performed at 190–400 nm. CD detection parameters were as follows: wavelength, 280 nm; response, STD; polarity, positive; gain, x10; UV range, short; threshold, 0.01 AU; CD range, 0.005°; scan speed, STD.

Results

LC– CD, LC–MS, and LC– MS/MS analysis

In LC–MS analysis, a peak corresponding to standard coronaridine at 15 min retention time was observed in the solid-phase extract of *C. roseus* (Figure 1). Although solid phase extraction was performed, alkaloids (e.g. vindoline and ajmaricine) with hydrophobicity equivalent to coronaridine were also detected. Further comparison of extracted ion chromatograms (m/z 339.20–339.21) revealed matching peaks at RT15 min for standard coronaridine and the *C. roseus* extract (Figure 2). No ions from other components were detected, confirming coronaridine as the main component of the RT15 min peak. LC–MS/MS analysis with CE values of 30, 50, and 60 confirmed that the MS/MS spectra of the *C. roseus* extract at RT15 min matched the standard coronaridine spectrum (Figure 3). Furthermore, it was estimated from accurate mass spectrometry that m/z 339.20643, estimated composition formula $C_{21}H_{27}O_2N_2$. These results suggested the presence of coronaridine in the leaves of *C. roseus*.

Methanol extraction of *C. roseus* leaves six weeks after sowing, followed by LC-CD analysis, revealed a negative peak in the *C. roseus* extract at the same retention time (RT14 min) as standard coronaridine (Figure 4). Additionally, CD spectrum measurement indicated matching CD spectra between standard coronaridine and the RT14 min peak of the *C. roseus* extract (Figure 5A). Furthermore, as a control, we measured the CD of catharanthine, which is known to have a (+) CD spectrum, and the CD spectrum was detected in the opposite direction to that of coronaridine extracted from *C. roseus* (Figure 5B). From the above results, Coronaridine contained in *C. roseus* was estimated to be (-) coronaridine.

Improved accumulation by high-temperature treatment

Typically, *C. roseus* is cultivated within a temperature range of 20 °C to 30 °C, with 25 °C considered optimal. In this experiment, the *C. roseus* under study was cultivated at the standard temperature of 25 °C. While investigating cultivation conditions to enhance coronaridine content during early growth stages, it was observed that cultivating at a temperature of 35 °C increased coronaridine levels in leaves. At 4 weeks of age, leaves from plants grown at 25 °C contained 30 mg g⁻¹ F.W. of coronaridine, while those from plants grown at 35 °C contained a significantly higher 320 mg g⁻¹ F.W. of coronaridine (Figure 6). Furthermore, a comparison of coronaridine content between upper and lower leaves of a single plant revealed that lower

leaves had a higher content, measuring $38.0 \mu\text{g g}^{-1}$ F.W., compared to $6.7 \mu\text{g g}^{-1}$ F.W. in upper leaves (Figure 7).

Analyzing the evolution of coronaridine content in leaves from seedlings at 2–9 weeks after sowing revealed the peak content at $580 \mu\text{g g}^{-1}$ F.W. at 3 weeks after sowing. Subsequently, as the number of days after sowing increased, coronaridine content gradually declined, reaching approximately 40 mg g^{-1} F.W. in plants at 9 weeks after sowing (Figure 8). Typically, flower buds emerge around the 9-week mark, and with further growth leading to pod formation, coronaridine becomes challenging to detect. This underscores the significant changes in coronaridine content dependent on the growth cycle. In investigating other alkaloids in *C. roseus*—catharanthine, vindoline, and ajmalicine—it was found that catharanthine (Figure 9A) and vindoline (Figure 9A) remained relatively constant irrespective of the growing season. Conversely, ajmalicine closely mirrored the change in coronaridine content, being abundant in young plants and diminishing as they matured. However, even as growth progressed, trace amounts of ajmalicine persisted and did not go unnoticed (Figure 9 C).

Discussion

This study aims to provide detailed insights into the accumulation of coronaridine in *C. roseus*. Referencing a report on coronaridine analysis in *T. iboga* (Farrow et al. 2019), we conducted LC–MS and LC–MS/MS analyses, discovering the presence of coronaridine in the methanol extract of *C. roseus* seedling leaves. Additionally, Farrow et al. (2019) noted the existence of (–) and (+) isomers of coronaridine in *T. iboga*, with *T. iboga* containing the (–) isomer. Our LC-DC analysis confirmed that coronaridine in *C. roseus* is of the (–) type, same to that in *T. iboga*. These findings underscore the biosynthesis of coronaridine in *C. roseus*, positioning it as a precursor within the metabolic pathway of iboga alkaloids.

It has been reported that the levels of the alkaloids catharanthine, vindoline, and vinblastine in the leaves of *C. roseus* are increased by high temperatures (Guo et al. 2007) and suppressed by low temperatures (Dutta et al. 2007, 2013). Therefore, when we compared the coronaridine content of *C. roseus* grown at 25 °C and at 35 °C, we found that the coronaridine content was significantly higher when grown at 35 °C than when grown at 25°C. Furthermore, when we analyzed changes in the amount of coronaridine accumulated depending on the growth stage, we found that the content of coronaridine contained per leaf weight was high in young plants, with the accumulated amount peaking in the 3rd to 4th weeks after sowing. During the subsequent growth period, the coronaridine content per plant remained constant, suggesting that the coronaridine content per leaf weight decreased as the plant grew. Until now, there have been few reports of the presence of coronaridine in *C. roseus* plants because mature plants have been used as research materials.

This study is the first to show that *C. roseus* contains coronaridine and that it is the same isomer as coronaridine found in *T. iboga*. It is expected that iboga alkaloids can be synthesized semisynthetically using coronaridine found in *C. roseus*.

Acknowledgements

This paper is based on the results of a project subsidized by the New Energy and Industrial Technology Development Organization (NEDO).

Author contribution

H.K. and T.M. conceived and designed the study. H.K. and H.H. cultivated and extracted. S.K. and T.O. analyzed alkaloids. M.I. managed the study.

Conflict of interest

The authors declare no conflict of interest.

References

- Bansal Y, Mujib A, Mamgain J, Dewir YH, Rihan HZ (2023) Phytochemical composition and detection of Novel bioactives in anther callus of *Catharanthus roseus* L. *Plants* 12: 2186.
- Benwell ME, Holtom PE, Moran RJ, Balfour DJ (1996) Neurochemical and behavioural interactions between ibogaine and nicotine in the rat. *Br J Pharmacol* 117: 743-749.
- Brown TK, Alper K. (2018) Treatment of Opioid Use Disorder with Ibogaine: Detoxification and Drug Use Outcomes. *Am. J. Drug Alcohol. Abuse* 44, 24–36. doi:10.1080/00952990.2017.1320802.
- Dhayanithy G, Subban K, Chelliah J (2019) Diversity and biological activities of endophytic fungi associated with *Catharanthus roseus*. *BMC Microbiol* 19: 1-14.
- Dutta A, Sen J, Deswal R (2007) Downregulation of terpenoid indole alkaloid biosynthetic pathway by low temperature and cloning of a AP2 type C-repeat binding factor (CBF) from *Catharanthus roseus* (L). G. Don. *G Don Plant Cell Rep* 26. *Plant Cell Rep* 26: 1869-1878.
- Dutta A, Sen J, Deswal R (2013) New evidences about strictosidine synthase (Str) regulation by salinity, cold stress and nitric oxide in *Catharanthus roseus*. *J Plant Biochem Biotechnol* 22: 124-131.
- Farrow SC, Kamileen MO, Meades J, Ameyaw B, Xiao Y, O'Connor SE (2018) Cytochrome P450 and *O*-methyltransferase catalyze the final steps in the biosynthesis of the anti-addictive alkaloid ibogaine from *Tabernanthe iboga*. *J Biol Chem* 293: 13821-13833.
- Farrow SC, Kamileen MO, Caputi L, Bussey K, Mundy JEA, McAtee RC, Stephenson CRJ, O'Connor SE (2019) Biosynthesis of an anti-addiction agent from the iboga plant. *J Am Chem Soc* 141: 12979-12983.
- Guo X, Yang L, Yu J, Tang Z, Zu Y (2007) Alkaloid variations in *Catharanthus roseus* seedlings treated by different temperatures in short term and long term. *J For Res* 18: 313-315.
- He DY, McGough NN, Ravindranathan A, Jeanblanc J, Logrip ML, Phamluong K, Janak PH, Ron D (2005) Glial cell line-derived neurotrophic factor mediates the desirable actions of the anti-addiction

drug ibogaine against alcohol consumption. *J Neurosci* 25: 619-628.

Hirosawa Y, Yasuda R, Minami W, Hirata M, Nugroho AE, Tougan T, Uchiyama N, Hakamatsuka T, Horii T, Morita H (2021) Divaricamine A, a new anti-malarial trimeric monoterpenoid indole alkaloid from *Tabernaemontana divaricata*. *Tetrahedron Letters* 83: 153423-153424.

Knuijver T, Schellekens A, Belgers M, Donders R, van Oosteren T, Kramers K, Verkes R (2022) Safety of ibogaine administration in detoxification of opioid-dependent individuals: a descriptive open-label observational study. *Addiction* 117: 118-128.

Mash DC, Duque L, Page B, Allen-Ferdinand K (2018) Ibogaine Detoxification Transitions Opioid and Cocaine Abusers between Dependence and Abstinence: Clinical Observations and Treatment Outcomes. *Front. Pharmacol.* 9, 529. doi:10.3389/fphar.2018.00529.

Mash DC (2018) Breaking the Cycle of Opioid Use Disorder with Ibogaine. *Am. J. Drug Alcohol Abuse* 44, 1–3. doi:10.1080/00952990.2017.1357184.

Mash DC, Kovera CA, Pablo J, Tyndale RF, Ervin FD, Williams IC, Singleton EG, Mayor M (2000). Ibogaine: Complex Pharmacokinetics, Concerns for Safety, and Preliminary Efficacy Measures. *Ann. N. Y. Acad. Sci.* 914, 394–401. doi:10.1111/j.1749-6632.2000.tb05213.x

Noller GE, Frampton CM, Yazar-Klosinski B (2018) Ibogaine treatment outcomes for opioid dependence from a twelve-month follow-up observational study. *Am J Drug Alcohol Abuse* 44: 37-46.

Rani S, Singh V, Sharma MK, Sisodia R (2021) GC-MS based metabolite profiling of medicinal plant- *Catharanthus roseus* under cadmium stress. *Plant Physiol. Rep.* doi: 10.1007/s 40502-021-00595-z

Santos RG, Bouso JC, Hallak JEC (2017) The Antiaddictive Effects of Ibogaine: A Systematic Literature Review of Human Studies. *J. Psychedelic Stud.* 1, 20–28. doi:10.1556/2054.01.2016.001.

Schenberg EE, de Castro Comis MA, Chaves BR da Silveira DX (2014) Treating Drug Dependence with the Aid of Ibogaine: A Retrospective Study. *J. Psychopharmacol.* 28, 993–1000. doi:10.1177/0269881114552713.

Singh B, Sharma RA, Vyas GK (2011) Antimicrobial, antineoplastic and cytotoxic activities of indole alkaloids from *Tabernaemontana divaricata* (L.) *R. Br. Curr Pharm Anal* 7: 125-132.

Wasko MJ, Witt-Enderby PA, Surratt CK (2018) DARK Classics in Chemical Neuroscience: Ibogaine. *ACS Chem. Neurosci.* 9, 2475–2483. doi:10. 1021/acscchemneuro.8b00294.

Figures Legends

Figure 1. Structural formula, molecular weight and composition formula of coronaridine, catharanthine, ajmalicine and vindoline

Figure 2. LC–MS measurement. A, standard coronaridine $10\ \mu\text{g ml}^{-1}$. B, *C. roseus* solid-phase extract. Arrow indicates coronaridine.

Figure 3. LC–MS measurement. MS spectrum. A, standard coronaridine $10\ \mu\text{g ml}^{-1}$. B, *C. roseus* solid-phase extract (Retention time, 15 min). Arrow indicates coronaridine.

Figure 4. LC–MS/MS measurement. MS/MS spectrum of peak detected at retention time 15 min of *C. roseus* extract. A, standard coronaridine $10\ \mu\text{g ml}^{-1}$. B, *C. roseus* solid-phase extract (Retention time, 15 min). Arrow indicates coronaridine.

Figure 5. LC-CD measurement. A and B, standard coronaridine $10\ \mu\text{g ml}^{-1}$. C and D, *C. roseus* solid-phase extract. A and C, CD chromatogram (280 nm). B and D, UV chromatogram (280 nm). Arrow indicates coronaridine.

Figure 6. CD measurement. A and B, standard coronaridine $100\ \mu\text{g ml}^{-1}$. C and D, *C. roseus* solid-phase extract (Retention time 14 min). E and F, standard catharanthine $100\ \mu\text{g ml}^{-1}$. A, C and E, CD chromatogram. B, D and F, UV chromatogram.

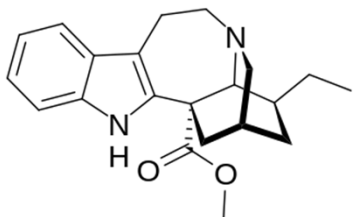
Figure 7. Coronaridine content in leaves of *C. roseus* 4 weeks after sowing grown at 25°C or 35°C ($N = 3$)

Figure 8. Coronaridine content in upper leaves and lower leaves of *C. roseus* 8 weeks after sowing grown at 25°C ($N = 3$).

Figure 9. Coronaridine content of *C. roseus* leaves from 2 to 9 weeks after sowing grown at 35 °C.

A, plant growth at each growth stage. B, coronaridine content in plant leaves at each stage (N = 3).

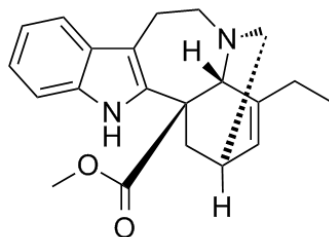
Figure 10. Contents of catharanthine, vindoline, ajmalicine in *C. roseus* leaves from 2 to 9 weeks after sowing grown at 35 °C (N = 3). A, catharanthine, B, vindoline and C, ajmalicine.



Coronaridine

Molecular weight: 338.44

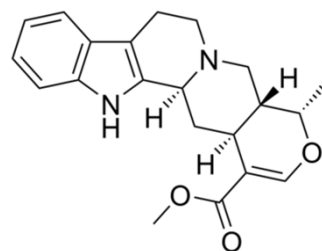
Composition formula: $C_{21}H_{26}N_2O_2$



Catharanthine

Molecular weight: 336.43

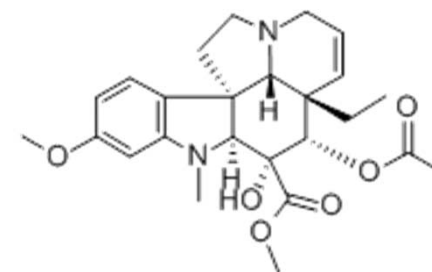
Composition formula: $C_{21}H_{24}N_2O_2$



Ajmalicine

Molecular weight: 352.43

Composition formula: $C_{21}H_{24}N_2O_3$



Vindoline

Molecular weight: 456.53

Composition formula: $C_{25}H_{32}N_2O_6$

Figure 1. Structural formula, molecular weight, and composition formula of coronaridine, catharanthine, ajmalicine and vindoline appearing in this study.

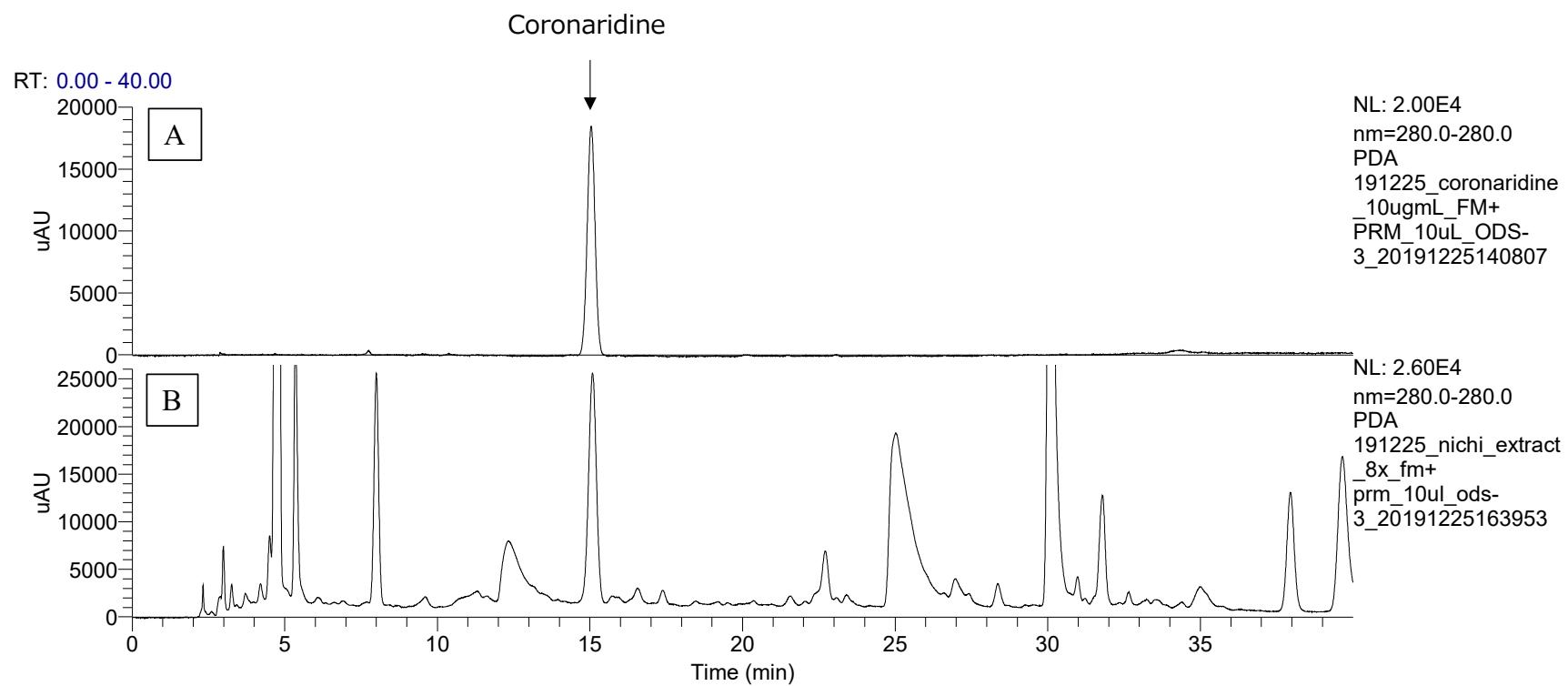


Figure 2. LC-MS measurement. A, Standard Coronaridine 10 μgml^{-1} . B, *C. roseus* solid phase extract. Arrow indicates coronaridine.

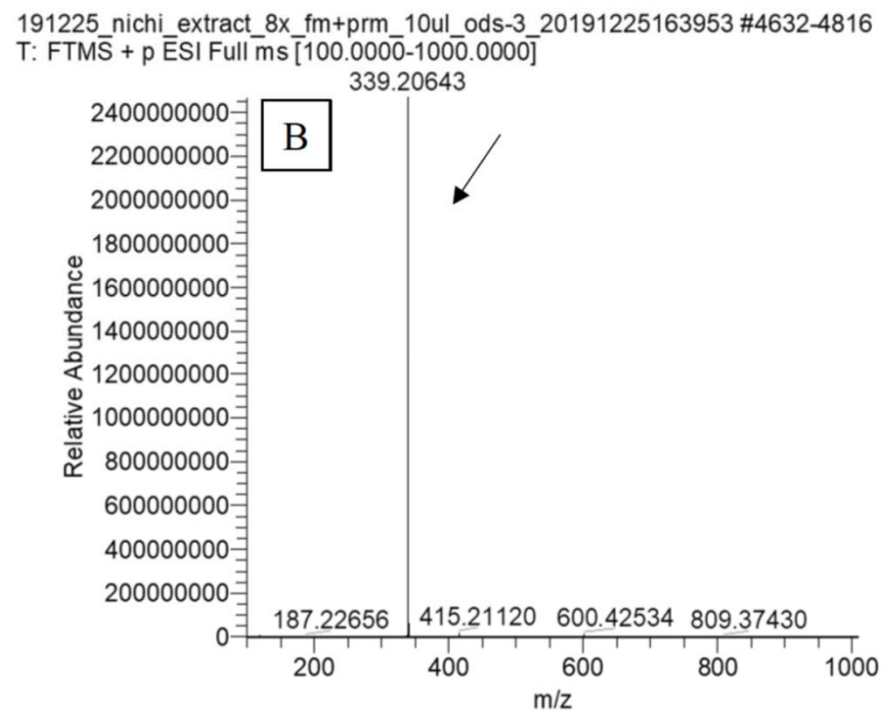
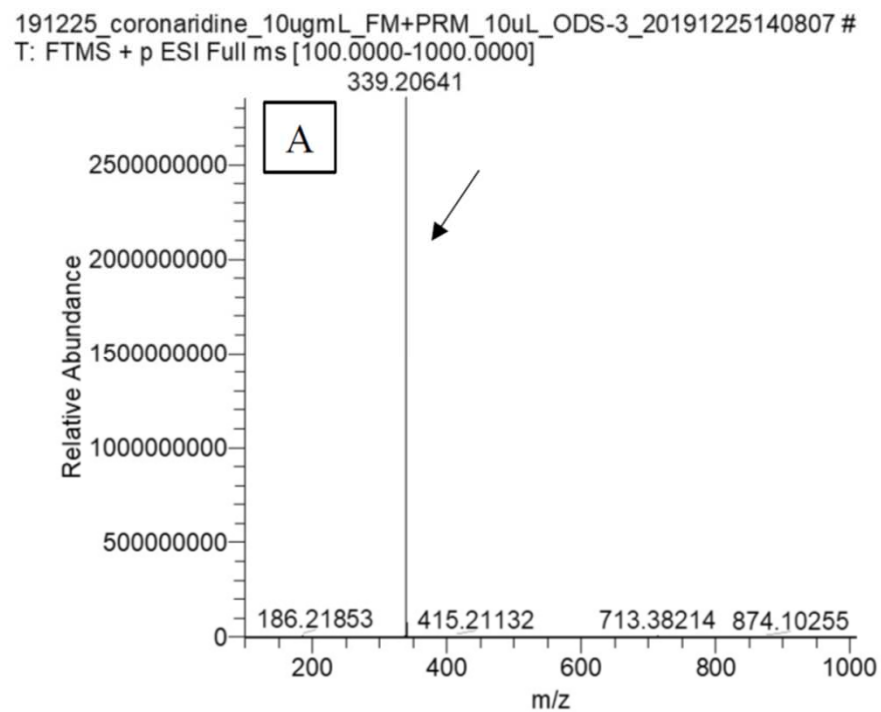


Figure 3. LC-MS measurement. MS spectrum. A, Standard coronaridine 10 μgml^{-1} . B, *C. roseus* solid phase extract (Retention time, 15 min). Arrow indicates coronaridine.

191225_coronaridine_10ugmL_FM+PRM_10uL_ODS-3_20191225140807 #4627-4752 RT: 14.86-15.21 AV: 9 SB: 191225_nichi_extract_8x_fm+prm_10ul_ods-3_20191225163953 #4660-4776 RT: 14.95-15.26 AV: 8 SB: 37
 F: FTMS + p ESI Full ms2 339.2064@hcd46.67 [50.0000-365.0000] F: FTMS + p ESI Full ms2 339.2064@hcd46.67 [50.0000-365.0000]

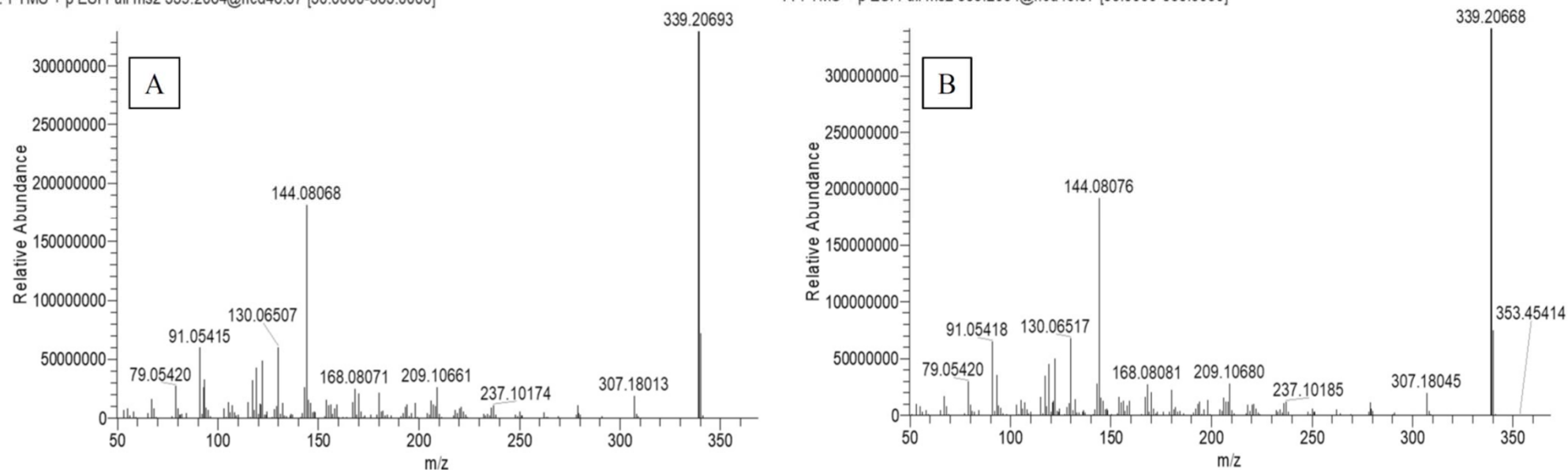


Figure 4. LC-MS/MS measurement. MS/MS spectrum of peak detected at retention time 15 min of *C. roseus* extract. A, standard coronaridine 10 µgml⁻¹. B, *C. roseus* solid phase extract (Retention time, 15 min).

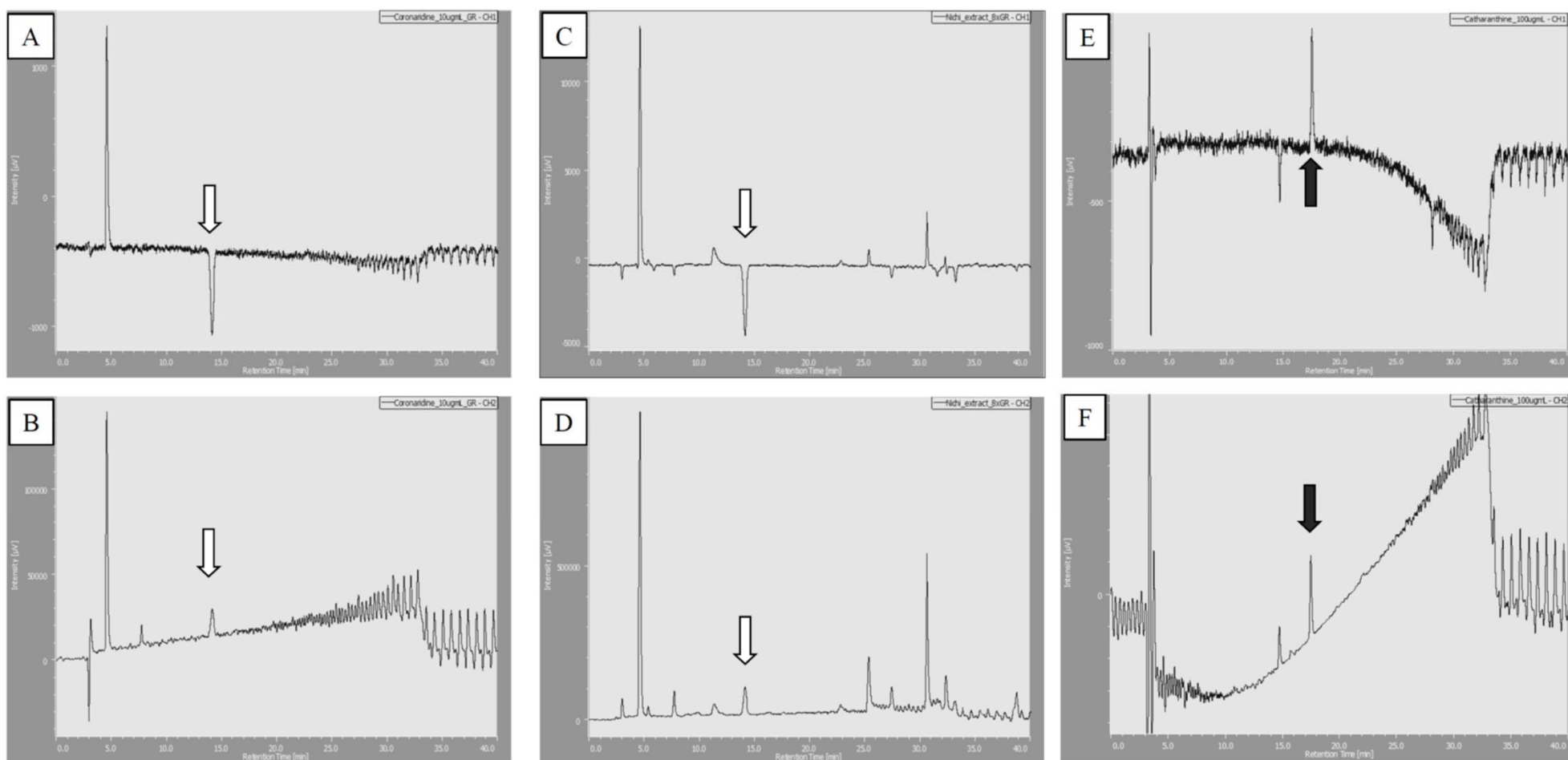


Figure 5. LC-CD measurement. A and B, standard coronaridine 10 μgml^{-1} . C and D, *C. roseus* solid phase extract. E and F, standard catharanthine 50 μgml^{-1} . A, C and E, CD chromatogram (280 nm). B, D and F, UV chromatogram (280 nm). White arrows indicate coronaridine. Black arrows indicate catharanthine.

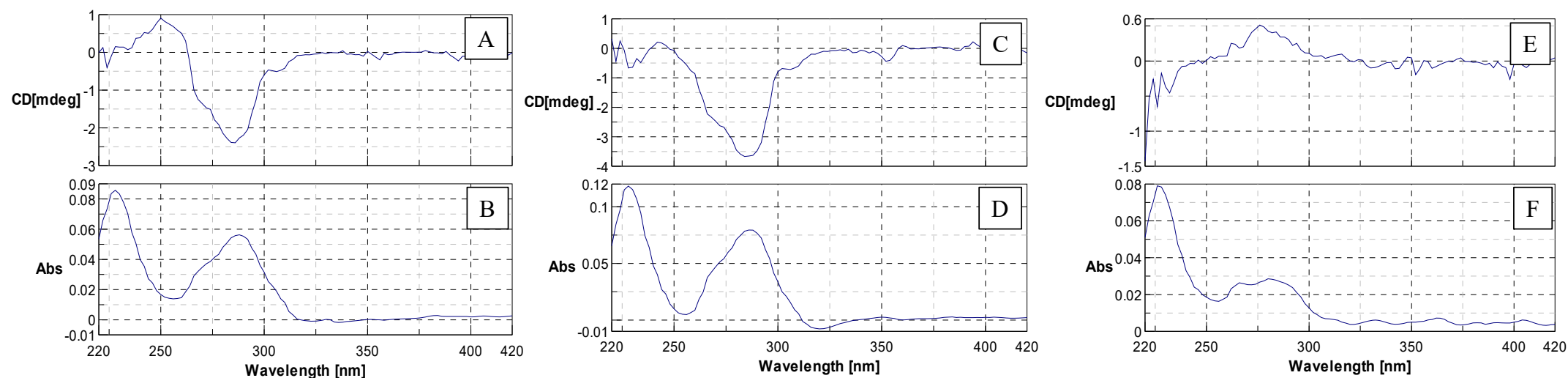


Figure 6. CD measurement. A and B, standard coronaridine $100 \mu\text{g ml}^{-1}$. C and D, *C. roseus* solid phase extract (Retention time, 14 min). E and F, standard catharanthine $50 \mu\text{g ml}^{-1}$. A and C, CD chromatogram. B and D, UV chromatogram.

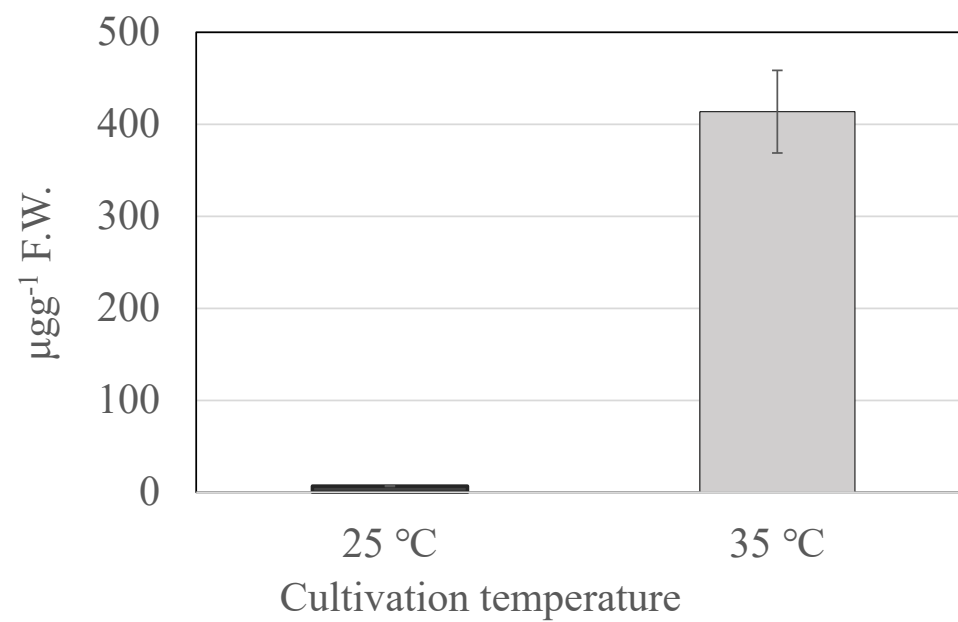


Figure 7. Coronaridine content in leaves of *C. roseus* 4 weeks after sowing grown at 25 °C or 35 °C (N = 3).

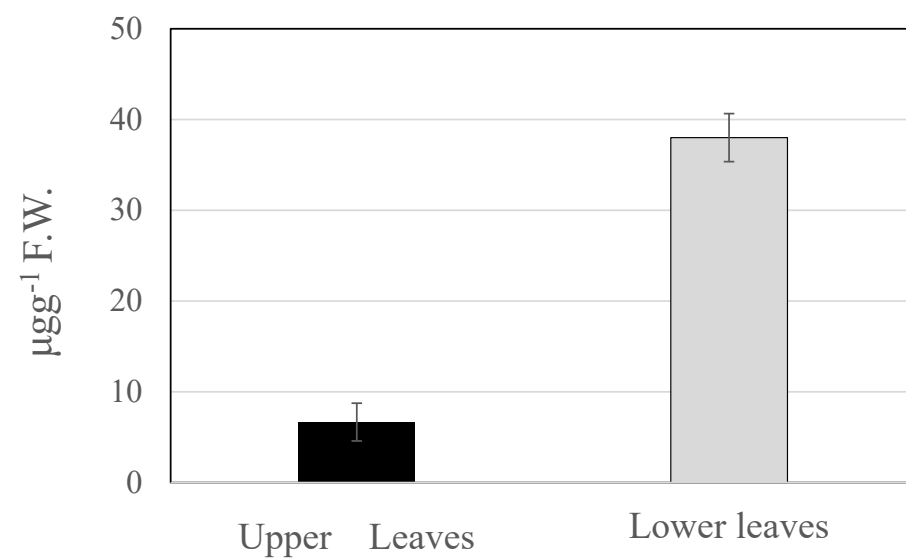


Figure 8. Coronaridine content in upper leaves and lower leaves of *C. roseus* 8 weeks after sowing grown at 25 °C (N = 3)

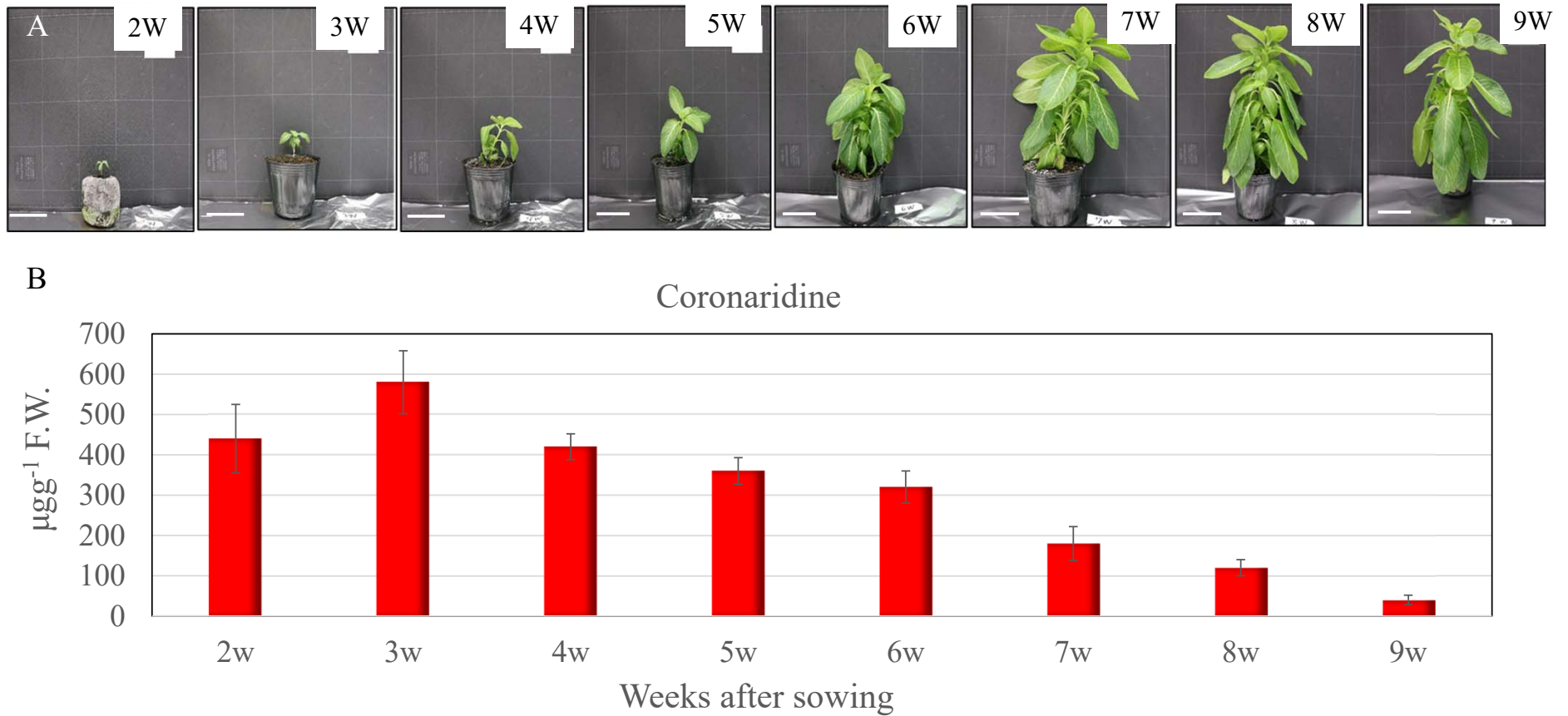


Figure 9. Coronaridine content of *C. roseus* leaves from 2 to 9 weeks after sowing grown at 35 °C. A, Plant growth at each growth stage. B, Coronaridine content in leaves of plants at each stage (N = 3). Bar: 5cm.

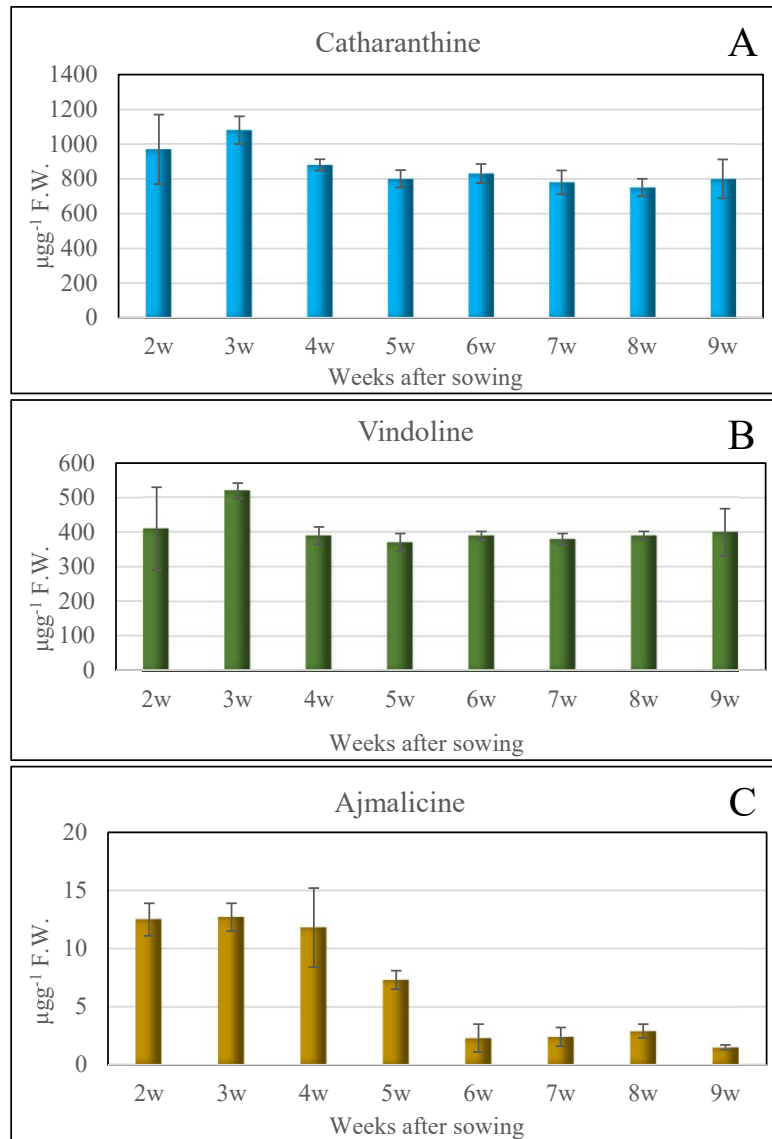


Figure 10. Contents of catharanthine (A), vindoline (B), ajmalicine (C) in *C. roseus* leaves from 2 to 9 weeks after sowing grown at 35 °C (N = 3).