Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
IL-17A inhibitions of indole alkaloids from traditional Chinese medicine Qing Dai

Chia-Lin Lee, Chien-Ming Wang, Yeh-Hsiung Kuo, Hung-Rong Yen, Ying-Chyi Song, Yu-Lun Chou, Chao-Jung Chen

*Corresponding author. Department of Cosmeceutics, China Medical University, Taichung, 40402, Taiwan.
E-mail addresses: chilee@mail.cmu.edu.tw (C.-L. Lee), magic2ming@yahoo.com.tw (C.-M. Wang), kuoyh@mail.cmu.edu.tw (Y.-H. Kuo), hungrongyen@gmail.com (H.-R. Yen), songyingchyi@gmail.com (Y.-C. Song), lulume770825@gmail.com (Y.-L. Chou), ironmanchen@yahoo.com.tw (C.-J. Chen).

1. Introduction

Qing Dai, a famous traditional Chinese medicine (TCM), is the pale blue to grayish-blue dried powder prepared by a traditional fermentation process (Lee et al., 2019; Lin, 2016; Pan et al., 2018). Currently, this TCM could treat various clinical inflammatory diseases, such as ulcerative colitis and psoriasis (Naganuma, 2019; Sugimoto et al., 2016). The aerial part of Strobilanthes cusia (Nees) Kuntze (Acanthaceae) recorded in Taiwan Herbal Pharmacopeia is one of the candidate materials that have been used to manufacture Qing Dai (Lee et al., 2019; Lin, 2016). The crude extracts and pure phytochemicals of S. cusia leave (Da-Ching-Yeh or Nan-Ban-Lan-Yeh) and root (Na-Ban-Lan-Gen) showed many pharmacological properties, including anti-viral (anti-herpes simplex virus type-1, anti-influenza A virus), anti-severe acute respiratory syndrome (SARS), antifungal, anti-inflammatory, antinociceptive, and antipyretic effects (Feng et al., 2016;...
Gu et al., 2015; Ho et al., 2003; Honda and Tabata, 1979; Tanaka et al., 2004; Zhou et al., 2017). To date many secondary metabolites, such as alkaloids, terpenoids, flavonoids, sterols, lignans, phanylethanoids, and isoocoumarin have been isolated from this species (Feng et al., 2016; Gu et al., 2015; Honda and Tabata, 1979; Tanaka et al., 2004; Zhou et al., 2017). However, interleukin 17 (IL-17) inhibitions of S. cusia and its processed drug, Qing Dai are not known. Qing Dai could provide a highly effective approach for not only psoriasis (topical use), but also inflammatory bowel disease (IBD) (oral use) in modern clinical therapy (Lee et al., 2019; Sugimoto et al., 2016). In addition, Qing Dai ointment could significantly reverse the IL-17A gene expression in psoriatic skin lesions in our previous studies (Cheng et al., 2017). Therefore, Qing Dai and its anti-IL-17 compounds are interesting for us and this TCM is bioactive constituents of Qing Dai resulted in isolation of two compounds; besides, their IL-17 inhibitory effects and possible biogenetic pathways of active components were also discussed within.

2. Materials and methods

2.1. Generals

1D and 2D NMR spectra were taken on Bruker Avance III 500 MHz. The chemical shift (δ) values are reported in ppm with CDCl3 used as the internal standard, and coupling constants (J) are in Hz. Low- and high-resolution ESIMS and EIMS were measured on a Bruker Daltonics Orbitrap mass spectrometer (LTQ Orbitrap XL and Q Exactive Plus, Thermo Fisher Scientific), respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. TLC was performed on Kieselgel 60 F254 (0.25 mm, Merck) and RP-18 Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively. Optical rotations, UV, and IR spectra were measured on a JASCO-P-2000 polarimeter (cell length 10 mm), a PerkinElmer®Lambdam265, and a Shimadzu model IR Prestige-21 Fourier-transform infrared spectrophotometer, respectively.

2.2. Plant material

The aerial parts of Strobilanthes cusia were collected in Putian City, Fujian Province, China and processed to TCM named Qing Dai by a local GMP pharmaceutical factory in April, 2014. The aforementioned TCM powders were imported and analyzed (Lot. No. BR0308980) by Sheng Chang Pharmaceutical Co., Ltd. in Zhongli District, Taoyuan City, Taiwan, and then was offered for this study in March, 2016. A voucher specimen (IN, 201603) was also stored at the CMRDC, CMUH, Taiwan (Lee et al., 2019).

2.3. Extraction and isolation

Qing Dai dry powders (10.0 kg) were extracted with MeOH (36 L × 4) at room temperature to obtain a crude extract (175.2 g) which was separated into an EtOAc-soluble fraction and an aqueous phase with EtOAc and H2O (1:1, v/v), respectively. The former one was further partitioned between n-hexane and 90% MeOH (aq) (1:1, v/v) to give the n-hexane- (INH, 74.8 g) and 90% MeOH (aq)- (INEA, 39.5 g) soluble crude fractions, individually. Fractionation of INEA fraction was conducted by open column chromatography on silica gel (column: 8.0 × 27 cm, diameter × length) using gradients of n-hexane/EtOAc/MeOH (10:1:0–0:1:1) to give eight subfractions (INEA-A–INEA-H). Subfraction INEA-F (3.8 g) was subjected to a silica gel column (3.5 × 37.5 cm; n-hexane/CH2Cl2/EtOAc, 2:1:0–0:0:1) to afford seven subfractions. Subfraction INEA-F-6 (1.4 g) was further separated by silica gel chromatography (column: 4.0 × 23.5 cm; n-hexane/EtOAc, 5:1-1:1) into nine fractions (INEA-F-6-1–INEA-F-6-9). The INEA-F-6-7 (340.3 mg) was purified by RP-18 column (3.0 × 22 cm; MeOH/H2O, 65:35 to 100:0) to obtain six subfractions (INEA-F-6-7-1–INEA-F-6-7-6). Subfraction INEA-F-6-7-2 (114.3 mg) was subjected to RP-18 gel chromatography (column: 3.0 × 22 cm; MeOH/EtOAc, 3:1) to obtain four subfractions. INEA-F-6-7-2-3 (64.4 mg) was further purified by silica gel chromatography (column: 2.5 × 25 cm; CH2Cl2 and column: 2.5 × 25.5 cm; n-hexane/EtOAc, 3:1) to give compound 2 (3.3 mg). Subfraction INEA-F-6-7-4 (29.6 mg) was further separated by HW-40F gel chromatography (column: 2.5 × 26.5 cm; CH2Cl2/Meth, 1:1), and its subfraction INEA-F-6-7-4-2 (10.9 mg) was isolated by silica gel chromatography (column: 2.5 × 26 cm; n-hexane/EtOAc, 3:1) to give compound 1 (8.3 mg).

2.3.1. Indigodole D (1)

Yellow powders; [α]D24 = -10.3 (c 1, MeOH); UV (MeOH) λ max nm (log e): 260 (4.18), 398 (3.74) (Fig. S8); IR (neat) νmax 3319, 2961, 2922, 2853, 2359, 2342, 1697, 1682, 1605, 1487, 1462, 1319, 1159, 1099, 1018, 1005, 988, 918, 885, 752, 667 cm⁻¹ (Fig. S9); For 1H and 13C NMR spectroscopic data, see Table 1; HRESIMS m/z 472.1629 [M + Na]+ (calcd for C28H33O2Na4Na, 472.1632).

2.4. HPLC system

A SHIMADZU LC-20AT (Kyoto, Japan) equipped with a degasser, a binary pump, a DAD detector SPD–M20A, and a liquid handler SIL–20A
autosampler was applied to analyze the chemical profile of Qing Dai. The mobile phase consisted of water containing 0.1% formic acid (eluent A) and acetonitrile (eluent B). The gradient program was used as following: 10%–76% B (22 min), 76%–85% (10 min), 85% isocratic elution (5 min), 85%–100% (5 min), 100% isocratic elution (8 min) with a HPLC column, COSMOSIL® 5C18–MS II (5 μm, 4.6 × 250 mm I.D.) performed at 25 °C. Before each analysis run, an equilibration time of 20 min was allowed. The flow rate was 1.0 mL/min and the injection volume was 20 μL. The pure compounds and crude extract concentrations were 0.1 and 1.0 mg/mL, respectively. UV/Vis spectra were recorded in the range of 200–800 nm.

2.5. T helper 17 (Th17) cell polarization and intracellular staining

Naïve CD4 T cells were isolated from spleens and peripheral lymph nodes harvested from C57BL/6 mice via EasySep™ Mouse T Cell Isolation Kit (STEMCELL Technologies Inc., Vancouver, Canada) according to the manufacturer’s protocol as previously described (Ben-Shaanan et al., 2018). The kit is designed to isolate T cells from single-cell suspensions of splenocytes and lymphocytes by negative selection. Unwanted cells are targeted for removal with biotinylated antibodies directed against non-T cells and streptavidin-coated magnetic particles. Desired CD4^+ T cells are isolated. 1 × 10^6 cells/well CD4^+ T cells were cultured in 96 well plate coated with 5 μg/mL anti-CD3 Ab for 5 days in the following polarization condition: 1 μg/mL anti-CD28 Ab (Biolegend), 20 ng/mL IL-6 (PeroTech), 1.25 ng/mL TGF-β (PeroTech), 20 ng/mL IL-1β (PeroTech), 20 ng/mL IL-23 (R&D, USA), 20 μg/mL anti-INF-γ Ab (Biolegend), and 20 μg/mL anti-IL-4 Ab (Biolegend). Culture medium used was IMDM (Gibco) supplemented with 1.0 mM sodium pyruvate (Gibco), 0.1 mM nonessential amino acids (Gibco), 100 IU/mL penicillin, 100 μg/mL streptomycin (Gibco), and 5% heat-inactivated FBS (Hyclone) (Harris et al., 2007; Yen et al., 2009).

For evaluation of the effect of Th17 polarization (“co-treated” experiment), naïve CD4^+ T cells were cultured with or without indicated compounds in the medium during the Th17 polarization for five days. For investigation of the level of IL-17A production after polarization (“post-treated” experiment), the polarized Th17 cells were treated with or without indicated compounds for 16 h (Lee et al., 2019).

After incubation, cells were restimulated for 5 h in the presence of PMA (50 ng/mL), ionomycin (500 ng/mL), and GolgiStop (BD Biosciences). The IL-17A secreting cells were stained with anti-CD4 Ab (BD Biosciences) and anti-IL-17A Ab (BioLegend) and analyzed by a BD FACSVerse® flow cytometer (Lee et al., 2019).

2.6. IL-17 luciferase reporter assay

The resultant IL-17 promoter-luciferase gene was cloned and constructed as described previously (Lee et al., 2019). Jurkat cells (4 × 10^6) were transfected with 10 μg IL-17 promoter-luciferase gene construct (pGL4-hIL-17prom) or control vector (pGL4.18) by electroporation according the manufacturer’s protocol (Neon transfection system, Invitrogen). Stable expression of IL-17 luciferase reporter from a clone of Jurkat cells were selected with G418 (800 μg/mL, GIBCO) for neomycin resistance. IL-17 luciferase reporter stably transfected cells (IL-17Luc cells) were seeded at the density of 1 × 10^5 cells/well in 96-well plate followed by treatment with PMA (50 ng/mL), ionomycin (500 ng/mL) and indicating compounds for 5 h. Assay medium was renewed and added a volume of Steady-Glo® Reagent equal to the volume of culture medium in the well for 30 min. Then the activity of luciferase in the transfected cells was measured with the BioTek Synergy microplate reader.

3. Results and discussion

3.1. Structure elucidation of compound 1

The MeOH extract of Qing Dai powders was partitioned into EtOAc- and H_2O-soluble extracts, and then the former one was further separated into n-hexane- and 90% MeOH-soluble fractions. Chromatographic fractionation of the active 90% MeOH-soluble one afforded new indigodole D (1) and known cephalandole B (2) (1D & 2D NMR spectra in supporting information) (Wu et al., 2006) (see Fig. 1). The molecular formula of 1 was deduced as C_{28}H_{22}O_{3}N_{3} due to the appearance of an [M+Na]^+ ion at m/z 472.1629 in the HRESIMS.
absorptions at 3319, 1697, and 1682 cm\(^{-1}\) supported the presence of NH and carbonyl groups, respectively. Other IR absorptions at 1605, 1487, and 1462 cm\(^{-1}\) indicated an aromatic system. The UV spectra showed absorption maxima at 260 and 398 nm. In 1D NMR, HSQC, and COSY data also indicated the presence of three sets of \(\beta\)-disubstituted benzene rings, one at \(\delta_{\text{H}}/\delta_{\text{C}}\) 6.31 (d, \(J = 7.5\) Hz)/114.6, 6.92 (t, \(J = 7.5\) Hz)/121.2, 7.33 (t, \(J = 7.5\) Hz)/136.5, 7.53 (d, \(J = 7.5\) Hz)/124.3, another at \(\delta_{\text{H}}/\delta_{\text{C}}\) 6.88 (t, \(J = 7.5\) Hz)/119.7, 6.94 (d, \(J = 7.5\) Hz)/112.1, 7.53 (d, \(J = 7.5\) Hz)/124.5, 7.59 (t, \(J = 7.5\) Hz)/139.6, and the other at \(\delta_{\text{H}}/\delta_{\text{C}}\) 6.74 (t, \(J = 7.5\) Hz)/118.1, 6.75 (d, \(J = 7.5\) Hz)/111.9, 7.39 (t, \(J = 7.5\) Hz)/137.8 and 7.60 (d, \(J = 7.5\) Hz)/124.7. The MS and NMR data of \(\text{Compound 2}\) showed absorption maxima at 260 and 398 nm.

3.2. Effects of isolates on IL-17A inhibition

Primary mouse CD4\(^+\) lymphocytes were polarized into Th17 cells to investigate the effect of two indole alkaloids 1 and 2 on Th17 polarization (co-treated experiment) and IL-17A secretion after Th17 polarization (post-treated experiment). In Fig. 4A, B, 5A and 5B, compounds 1 and 2 were co-cultured with CD4\(^+\) lymphocytes in the skewing medium for five days to evaluate the effect of isolates on Th17 polarization (co-treated experiment). Intracellular staining with flow cytometric analysis (Fig. S17A) showed that 1 reduced the polarization of IL-17A secretion cells (EC50 value = 2.16 \(\mu\)g/mL) without significant cytotoxicity to T cells (Fig. 4A and B). However, compound 2 co-treatment did not significantly inhibit IL-17A polarization in a dose-dependent manner (Fig. 5A and B and Fig. S17B). In Fig. 5B, although it seemed that compound 2 could inhibit IL-17A protein production at concentrations from 0.2 to 1.56 \(\mu\)g/mL in a dose-dependent manner; however, this dose-dependent inhibitory effect was not seen at concentrations from 3.125 to 12.5 \(\mu\)g/mL.

Moreover, we investigated the level of IL-17A production from Th17 cells by adding compounds 1 and 2 for 16 h after Th17 polarization (post-treated experiment) (Fig. 4C, D, 5C and 5D). Compound 1 could significantly inhibit IL-17 production (EC50 value = 5.99 \(\mu\)g/mL) without cytotoxicity to Th17 cells (Fig. 4C and D). However, the
decrease of IL-17A protein production of compound 2 in the post-treated experiment (Fig. 5D) might be due to the cytotoxicity to Th17 cells at 25 and 50 μg/mL concentrations (Fig. 5C). Besides, it has to be mentioned that in the IL-17 luciferase reporter assay, both compounds 1 and 2 could notably inhibit the IL-17 gene expression in Jukat cells (immortalized T lymphocytes) which were transfected with IL-17 luciferase reporters (Fig. 6).

In our previous study, indole alkaloids, including indigodole A (3), indigodole C (4), tryptanthrin (5), and indirubin (6) were also suggested as anti-IL-17 contributors of Qing Dai (Lee et al., 2019). Those active components were applied to chemical profile analyses using HPLC/PDA chromatograms. As shown in Fig. 7, the HPLC chemical analysis of this TCM methanolic extract indicated active compounds 1–6 at retention times of 21–27 min (Table S1).
3.3. Plausible biogenetic pathways for active indole alkaloids of Qing Dai

Qing Dai is manufactured by a traditional process from indigo plants such as *S. cusia*. The fresh botanical aerial portion containing leaves with stems are immersed in water to rot by the enzymes from itself or microorganisms. After that, the tissue residues are taken out and lime (CaCO₃) is added. The mixture is constantly stirred that makes oxygen in air to take part in. Consequently, a lot of foam on the liquid surface will be generated, collected, and further dried to prepare the pale blue to grayish-blue powder that is used as TCM named Qing Dai (Lee et al., 2019; Lin, 2016; Pan et al., 2018).

It is possible to describe the biosynthetic origins of indole alkaloids of Qing Dai on the basis of three major factors, precursor indole components from *S. cusia*, oxygen in air, and alkali condition. In the proposed mechanisms (Figs. 8, 9, S18), indole will be oxidized to indolone, cytochrome P-450 (Cyt P-450) is a monooxygenase for oxidation, acetyl-CoA could offer ethyl group, and NADPH (nicotinamide adenine dinucleotide phosphate) will give hydrogen for reduction reaction. The
plausible biogenetic mechanisms of active indigodoles A, C, and D are shown in Figs. 8 and 9, respectively, in addition, those of indirubin, tryptanthrin, and indigodole B, that a tryptanthrin derivative without anti-IL-17 property in our previous investigation, are shown in Fig. S18.

4. Conclusions

Qing Dai, a processed drug from indigo plants, has been used in modern clinical therapy for many inflammation diseases. But their active components were reported only rarely and we believe they are worthy of more investigations. In this study, two indole alkaloids, including new indigodole D (1), were obtained from Qing Dai and both isolates showed inhibition against IL-17A gene expression, especially I also showed dose-dependent inhibition on IL-17A protein production. Besides, indole alkaloids indigodole A, indigodole C, tryptanthrin, and indirubin could contribute to the anti-IL-17A properties of Qing Dai as well. The possible biogenetic mechanisms of above-mentioned indoles were also speculated by us and could provide valuable information for medicinal chemists to make more active Qing Dai indole analogues. Structure-activity relationships and IL-17 mechanism of action will be needed to further evaluate and clearly clarify for further clinical application.

Author contributions

Conceived and designed the experiments: CLL and HRY. Performed the experiments: CLL, CMW, YCS, YLC, CJIC. Analyzed the data: CLL, YHK, HRY, CJIC. Wrote the paper: CLL, YHK, HRY, YCS.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This work was financially supported by the “Chinese Medicine Research Center, China Medical University” from The Featured Areas Research Center Program within the framework of the Higher Education Sprout Project by the Ministry of Education (MOE) in Taiwan (CMRC-CHM-2-1). The authors deeply appreciated to Sheng Chang Pharmaceutical Co., Ltd to offer authentic materials for this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.112772.

References

Ben-Shaanan, T.L., Schiller, M., Azulay-Debby, H., Korin, B., Boshnak, N., Koren, T., Krot, M., Shaka, J., Rahat, M.A., Hakim, F., Rolls, A., 2018. Modulation of anti-tumor immunity by the brain’s reward system. Nat. Commun. 9, 2723.

Cheng, H.M., Wu, Y.C., Wang, Q., Song, M., Wu, J., Chen, D., Li, K., Wadman, E., Yao, S.T., Li, T.C., Leon, F., Hayden, K., Brodmerek, C., Chris Huang, C., 2017. Clinical efficacy and IL-17 targeting mechanism of Indigo Naturalis as a topical agent in moderate psoriasis. BMC Compl. Alternative Med. 17, 439.

Feng, Q.T., Zhu, G.Y., Gao, W.N., Yang, Z., Zhong, N., Wang, J.R., Jiang, Z.H., 2016. Two new alkaloids from the roots of Baphicacanthus cusia. Chem. Pharm. Bull. 64, 1505–1508.

Gu, W., Wang, W., Li, X.N., Zhang, Y., Wang, L.P., Yuan, C.M., Huang, L.J., Hao, X.J., 2015. A novel isocoumarin with anti-influenza virus activity from Sirobalanthes cusia. Fitoterapia 107, 60–62.

Harris, T.J., Grosso, J.F., Yen, H.R., Xin, H., Kortylewski, M., Albesiano, E., Hipkiss, E.L., Getnet, D., Goldberg, M.V., Maris, C.H., Housseau, F., Yu, H., Pardoll, D.M., Drake, C.G., 2007. Cutting edge: an in vivo requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. J. Immunol. 179, 4313–4317.

Ho, Y.L., Kao, K.C., Tsai, H.Y., Chueh, F.Y., Chang, Y.S., 2003. Evaluation of anti-nociceptive, anti-inflammatory and antipyretic effects of Sirobalanthes cusia leaf extract in male mice and rats. Am. J. Chin. Med. 31, 61–69.

Honda, G., Tabata, M., 1979. Isolation of antifungal principle tryptanthrin, from Sirobalanthes cusia O. Kuntze. Planta Med. 36, 85–90.

Lee, C.L., Wang, C.M., Hu, H.C., Yen, H.R., Song, Y.C., Yu, S.J., Chen, C.J., Li, W.C., Wu, Y.C., 2019. Indole alkaloids indigodoles A-C from aerial parts of Sirobalanthes cusia in the traditional Chinese medicine Qing Dai have anti-IL-17 properties. Phytochemistry 162, 39–46.

Lin, T.Y., 2016. Taiwan Herbal Pharmacopoeia, second ed. Ministry Health and Welfare, Taipei, Taiwan, R.O.C, pp. 186–187 English version.

Naganuma, M., 2019. Treatment with indigo naturalis for inflammatory bowel disease and other immune diseases. Immunol. Med. 42, 16–21.

Pan, M., Pei, W., Yao, Y., Dong, L., Chen, J., 2018. Rapid and integrated quality assessment of organic-inorganic composite herbs by FTIR spectroscopy-global chemical fingerprints identification and multiple marker components quantification of Indigo Naturalis (Qing Dai). Molecules 23, 2743.

Sugimoto, S., Naganuma, M., Kanai, T., 2016. Indole compounds may be promising medicines for ulcerative colitis. J. Gastroenterol. 51, 853–861.

Tanaka, T., Ikeda, T., Kaku, M., Zhu, H.X., Okawa, M., Yokonoto, K., Ueda, M., Nohara, T., 2004. A new lignan glycoside and phenylethanoid glycosides from Sirobalanthes cusia BREMEK. Chem. Pharm. Bull. 52, 1242–1245.

Wu, P.L., Hsu, Y.L., Jao, C.W., 2006. Indole alkaloids from Cephalanceropsis gracilis. J. Nat. Prod. 69, 1467–1470.

Yen, H.R., Harris, T.J., Wada, S., Grosso, J.F., Getnet, D., Goldberg, M.V., Liang, K.L., Bruno, T.C., Pyle, K.J., Chao, S.I., Anders, R.A., Trimble, C.L., Adler, A.J., Lin, T.Y., Pardoll, D.M., Huang, C.T., Drake, C.G., 2009. Tc17 CD8 T cells: functional plasticity and subset diversity. 183, 7161–7168.

Zhou, B., Yang, Z., Feng, Q., Liang, X., Li, J., Zanin, M., Jiang, Z., Zhong, N., 2017. Aurantiamide acetate from Sirobalanthes cusia root exhibits anti-inflammatory and anti-viral effects via inhibition of the NF-kB signaling pathway in influenza A virus-infected cells. J. Ethnopharmacol. 199, 60–67.