

# Bioactive constituents from *Linaria japonica* and *Spilanthes acmella*

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## Introduction

Natural products from medicinal plants provide unlimited opportunities for new drug discovery. Due to an increasing demand for chemical diversity, seeking therapeutic drugs from natural products have grown throughout the world. *Linaria japonica* is a perennial herb that grows in seashore area and used in folk medicine as diuretic and purgative. *Spilanthes acmella* is an important medicinal plant, found in tropical and subtropical countries. The extracts and compounds from this plant possess useful pharmacological activities.<sup>1)</sup> On our activity screening of various plants, we found anti-leishmanial and osteoblast stimulatory activities from *L. japonica* and *S. acmella*, respectively. Therefore we performed phytochemical studies for identifying active ingredients.



*L. japonica*



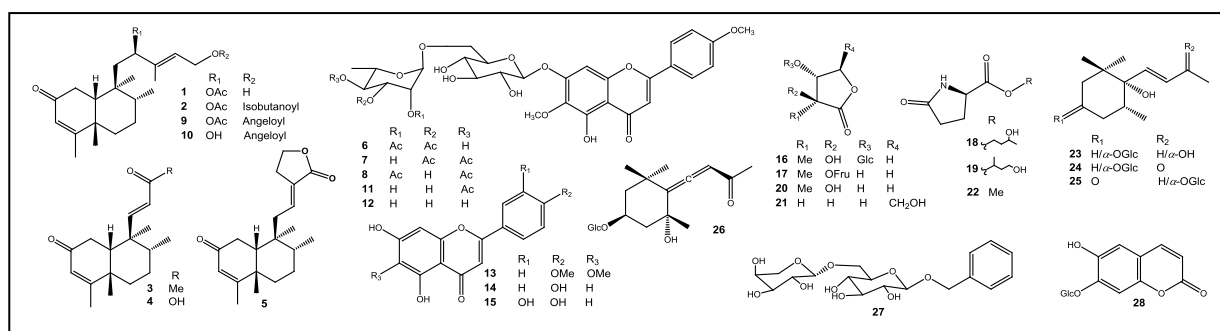
*S. acmella*

## Materials and Methods

The whole plants of *Linaria japonica* and *Spilanthes acmella* were collected from Tottori (Japan) and Purwodadi (Indonesia), respectively. The air-dried plants were extracted with methanol, and then partitioned with *n*-hexane, ethyl acetate and butanol, successively. The mixture of *n*-hexane and ethyl acetate layer of *Linaria japonica* and butanol layer of *Spilanthes acmella* were separated on various chromatographic techniques. The chemical structures of the isolated compounds were determined by spectroscopic and chemical analyses. The inhibitory activity against *Leishmania major* parasite is evaluated by MTT method. Alkaline phosphatase (ALP) and mineralization stimulatory activities of MC3T3-E1 cell were investigated as osteoblast markers. Other pharmacological activities including cytotoxicity against A549 cell, collagenase, and advanced glycation end products (AGEs) formation were also evaluated in this study.

## Results and Discussion

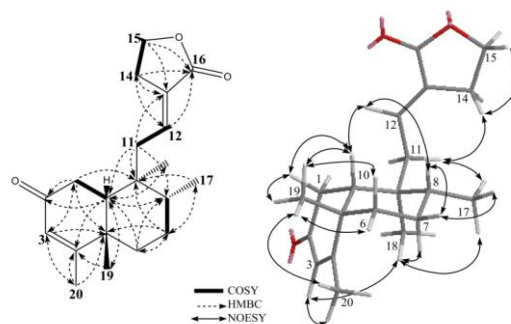
The mixture of *n*-hexane and ethyl acetate layer of whole plant of *Linaria japonica* led to the isolation of eight new compounds (1–8) and seven known compounds (9–15). The butanol layer of *Spilanthes acmella* contains four new compounds (16–19) and nine known compounds (20–28) (Fig. 1). These chemical structures were determined by various spectroscopic analyses and chemical evidences (Fig. 2 and 3).



**Figure 1.** Isolated compounds of *Linaria japonica* and *Spilanthes acmella*

### Structural analysis of **5**

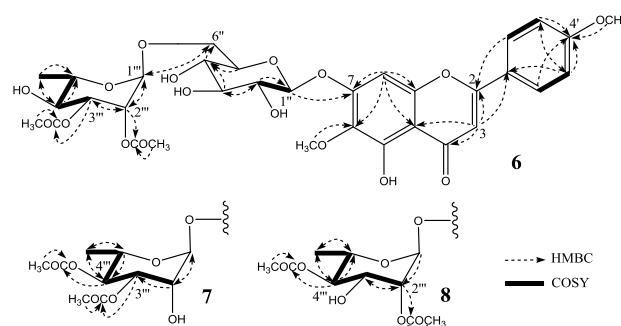
The molecular formula of **5** was assigned as  $C_{20}H_{28}O_3$  by HR-ESI-MS at  $m/z$  339.1929  $[M+Na]^+$  (calcd 339.1931). The IR absorption band at  $1754\text{ cm}^{-1}$  indicated  $\gamma$ -lactone moiety. The  $^1H$  and  $^{13}C$  NMR spectra revealed a *cis*-clerodane framework with a typical  $\gamma$ -lactone ring [ $\delta_H$  2.86 (br t,  $J = 7$  Hz),  $\delta_H$  4.37 (t,  $J = 7$  Hz) and  $\delta_C$  25.6, 65.6, 127.4, 171.3] and a six membered ring ketone [ $\delta_C$  198.7]. The  $\gamma$ -lactone was connected to C-12 according to the HMBC correlations of H-12 to C-16 and C-14, together with the correlations of H-11, 14 and 15 to C-13. The NOESY experiment revealed its relative structure as a *cis*-clerodane framework (Fig. 2). Finally the absolute stereochemistry of **5** was established as *5S, 8R, 9S* and *10R* by means of the CD spectrum ( $\Delta\epsilon +1.13$  at 327 nm). Accordingly, the structure of **5** was determined as (*5S, 8R, 9S, 10R*)-2-oxo-*cis*-cleroda-3*Z, 12E*-dien-15,16-olide.<sup>2)</sup>



**Figure 2.** 2D NMR analysis of **5**

### Structural analysis of **6-8**

The molecular formula of  $C_{33}H_{38}O_{17}$  (**6**) were determined by HR-ESI-MS at  $m/z$  729.1998  $[M+Na]^+$  (calcd. 729.2001).  $^1H$  and  $^{13}C$  NMR spectrum of **6** displayed a methyl of rhamnose moiety [ $\delta_H$  1.17 (d,  $J = 6.2$  Hz),  $\delta_C$  18.0], two methyl of acetyl group [ $\delta_H$  1.75 (s), 1.93 (s),  $\delta_C$  20.6, 20.8, 171.5, 172.1], two methoxy groups [ $\delta_H$  3.89 (s),  $\delta_C$  56.2, 61.7], and two anomeric signals of sugars [ $\delta_H$  4.72 (br s), 5.19 (d,  $J = 7.2$  Hz),  $\delta_C$  99.3, 101.6]. The acetyl substituents were placed at C-2'' and 3'' on the basis of the HMBC correlations of H-2'' and 3'' to C-2'' OAc and 3'' OAc, respectively. The D-glucose moiety was connected to C-7 according to the HMBC correlations of H-1'' to C-7, and the L-rhamnose moiety was connected to C-6'' according to their correlations of H-1''' to C-6''. The HMBC spectrum exhibited correlations between H-3 to C-1', C-2 and C-4, which follow us to assign this proton at position 3 of the flavone core (Fig. 3). The structure of **6** was determined as pectolarigenin-7-*O*-(2,3-diacetyl- $\alpha$ -L-rhamnopyranosyl)-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranoside. Compound **7** and **8** have different position of acetoxy group at rhamnose moiety.<sup>3)</sup>



**Figure 3.** 2D NMR analysis of **6-8**

## Biological activities of the isolated compounds

### Anti-*Leishmania*, AGEs and collagenase activities of *L. japonica*

All the isolated compounds of *Linaria japonica* (1–15) were evaluated the inhibitory activities against *L. major* parasites. The compounds 1, 9 and 10 showed moderate inhibition of *L. major* (IC<sub>50</sub> value of 56.7±1.8, 50.3±1.6 and 52.7±2.6 μM, respectively), and compounds 2 and 5 found to be active (IC<sub>50</sub> value of 89.4±7.2 and 97.3±5.9 μM, respectively). It is noteworthy that 1 and 10 were relatively selective against *L. major* than cytotoxicity (IC<sub>50</sub> value of >100 μM) (Table 1). Compounds 6, 7, and 8 showed stronger AGEs formation inhibitory activity, and compounds 11 and 12 have moderate collagenase inhibitory activities, without any significant cytotoxicity (Table 2). These activity of the extract and isolated compounds are clarified for the first time in this study.<sup>2,3)</sup>

### Osteoblast stimulatory activity of *S. acmella*

To investigate whether the isolated compounds of *S. acmella* (16–28) could stimulate the function of osteoblasts, alkaline phosphatase (ALP) and mineralization activities were assessed on the pre-osteoblastic cell line, MC3T3-E1, which has been a well-characterized as an *in vitro* model for osteoblast differentiation. New compound 19, 2–deoxy-D-ribo-1,4 lactone (21), ampelosisinoside (25), and benzyl-α-L-arabinopyranosyl (1→6)-β-D-glucopyranoside (27) stimulated alkaline phosphatase and mineralization activity up to 112%, which were comparable to the positive control, 17β-estradiol (110% and 106%, respectively) without any significant cytotoxicity (Fig. 4). This study provides further usefulness of this plant material as the osteoblast stimulating activity toward osteoporosis.

**Table 1.**  
Anti-*Leishmania* activity of isolated compounds from *L. japonica*

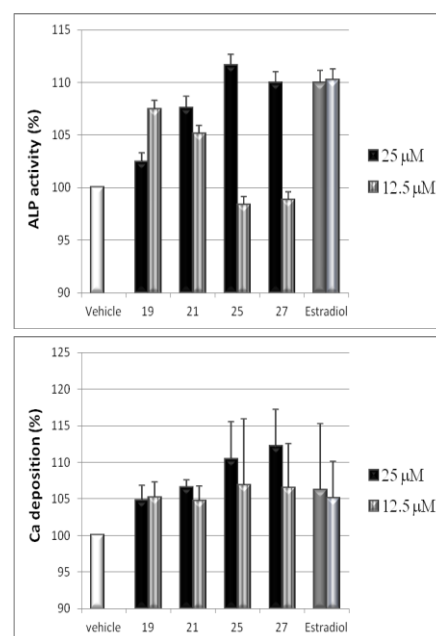
Isolated Compounds	A549 (IC <sub>50</sub> , μM)	<i>L. major</i> (IC <sub>50</sub> , μM)
New compound 1	–	56.7±1.8
New compound 2	–	89.4±7.2
New compound 3	51.2±2.6	–
New compound 4	–	–
New compound 5	86.5±5.7	97.3±5.9
New compound 6	–	–
New compound 7	–	–
New compound 8	–	–
Linarienone (9)	79.0±9.9	50.3±1.6
Desacetyl-linarienone (10)	–	52.7±2.6
Linariin (11)	–	–
Pectolarin (12)	–	–
Pectolarigenin (13)	91.1±6.7	–
Apigenin (14)	–	–
Luteolin (15)	82.6±5.4	77.7±5.4
Doxorubin	0.7±0.06	n.d
Miltefosine	n.d	17.8±1.1

– : > 100 μM, n.d: not determined

**Table 2.**  
Anti-AGEs and collagenase activities of flavonoids from *L. japonica*

Isolated Compounds	AGEs (IC <sub>50</sub> , μM)	Collagenase (IC <sub>50</sub> , μM)
New compound 6	34.8±5.6	–
New compound 7	35.0±8.8	–
New compound 8	19.5±2.0	–
Linariin (11)	–	79.4±3.8
Pectolarin (12)	–	78.6±2.4
Pectolarigenin (13)	–	–
Apigenin (14)	–	–
Luteolin (15)	85.8±6.8	40.5±3.2
Aminoguanidine	1290±31.5	n.d
Caffeic acid	n.d	120±1.8

– : > 100 μM, n.d: not determined



**Figure 4.** The osteoblast stimulatory activity

### References:

- 1) Phytochemistry, pharmacology and toxicology of *Spilanthes acmella*: A Review. Dubey S. *et al. Adv Pharmacol Sci.* (2013), Article ID 423750
- 2) New *cis-ent*-clerodanes from *Linaria japonica*. Retno W. *et al. Phytochem. Lett.* **14**, 56– 62 (2015)
- 3) New isolinariins C, D and E, flavonoid glycosides from *Linaria japonica*. Retno W. *et al. Chem. Pharm. Bull.* **64**, 517-521 (2016)