



Original Article

## Antibacterial and anti-HIV-1 integrase properties of isolated compounds from *Boesenbergia kingii*

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### Abstract

*Boesenbergia kingii* Mood & L.M. Prince, a medicinal plant used to treat infected inflammatory disease was investigated for antibacterial and anti-HIV-1 integrase (IN) activities using the broth dilution method and multiplate integration assay, respectively. Seven compounds were isolated from the active chloroform fraction of *B. kingii* including 8-hydroxy-dauca-9,11-diene-7-one (**1**), dauca-8,11-diene-7-one (**2**), dauca-8,11-diene-7,10-dione (**3**), kaempferol-7,4'-dimethyl ether (**4**), kaempferol-3,7,4'-trimethyl ether (**5**), dihydrobisdemethoxycurcumin (**6**) and bisdemethoxycurcumin (**7**). All of sesquiterpenes markedly exhibited the inhibitory activity against *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922. Compound **6** exhibited the highest activity against only *S. aureus*. However, the most active antibacterial **6** did not inhibit the activity of HIV-1 IN whereas **7** showed moderate anti-HIV-1 IN and antimicrobial activities. This study demonstrated that some compounds found in *B. kingii* are responsible for anti-bacterial activity and anti-HIV-1 IN. The results of this study support the ethnomedicinal uses of *B. kingii* to some extent.

**Keywords:** *Boesenbergia kingii*, antibacterial activity, anti-HIV-1 integrase, sesquiterpenes, flavonoids

### 1. Introduction

The increasing of HIV/AIDS infected patients and the high prevalence rate of opportunistic infections caused by the acquired immune deficiency are the major public health problem. Moreover, the increasing of multidrug resistant by pathogenic microbes in AIDS patients is of concern. These infections are the leading cause of the death of AIDS patients worldwide as well as in many developing countries (Tchouya *et al.*, 2015). In some countries, indigenous medicinal plants are used throughout history to cure and prevent many ill-

nesses by traditional healer. In this situation, medicinal plants are used as primary treatment for HIV-related problems such as skin disorders, nausea, depression, insomnia, and body weakness. In addition, the traditional healers also demonstrated the relevance of herbal therapies in the treatment of HIV/AIDS opportunistic diseases (Chinsemu & Hedimbi, 2010).

The rhizome of *Boesenbergia longiflora* (Wall.) Kuntze, a plant in *Boesenbergia* genus has been used for treatment of infected inflammatory related ailments including inflammatory bowel disease, ulcerative colitis and abscess by Thai traditional healers. *B. longiflora* has been re-identified by phylogenetic analysis and nuclear ITS DNA sequence data as *Boesenbergia kingii* Mood & L.M. Prince (Mood *et al.*, 2013). As a part of our study on anti-inflammatory

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agents from *B. kingii* rhizomes, it was indicated that the chloroform fraction of this plant possesses potent anti-inflammatory activity as well as wound-healing activity (Sudsai *et al.*, 2013). Diarylhepanoids, flavonoids and sesquiterpene, the active constituents isolated from *B. kingii*, exhibited anti-inflammatory activity in both *in vitro* and *in vivo* studies (Sudsai *et al.*, 2014). Due to these properties, *B. kingii* has gained attention as an important source for medicinal treatment. However, studies describing the pharmacological properties of *B. kingii* on the microbial infections and HIV-1 had still not been reported.

## 2. Materials and Methods

### 2.1 Plant material

The fresh rhizomes of *Boesenbergia kingii* were bought from Chatuchak weekend market in Bangkok, Thailand, in June 2010 and identified by Dr. John D. Mood, Lyon Arboretum, University of Hawaii, USA. The voucher specimen (SKP2060200-101) is deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand.

### 2.2 Preparation of plant extracts and isolation

Based on our previous study, plant material were dried at 50°C, powdered and macerated with 95% ethanol, then concentrated to dryness under reduced pressure. The ethanolic extract obtained from the extraction was further partitioned into 4 fractions including hexane, chloroform (CHCl<sub>3</sub>), ethyl acetate (EtOAc) and water fractions. Then, the antibacterial assay was performed and the CHCl<sub>3</sub> fraction significantly exhibited potent antibacterial activity (MIC/MBC = 16/36 µg/ml). Therefore, the CHCl<sub>3</sub> fraction was then subjected to bioassay-guided isolation and seven compounds (1-7, Figure 1) were obtained. These compounds were elucidated by comparison with spectral data (<sup>1</sup>H NMR, <sup>13</sup>C NMR and MS) of our previously report (Sudsai *et al.*, 2014). The isolated

compounds could be classified into three groups, sesquiterpenes (1, 2 and 3), flavonoids (4 and 5) and diarylheptanoids (6 and 7). The structures of isolated compounds were elucidated by spectroscopic methods as 8-hydroxy-dauca-9,11-diene-7-one (longiferone A: 1), dauca-8,11-diene-7-one (longiferone B: 2) and dauca-8,11-diene-7,10-dione (longiferone C: 3), kaempferol-7,4'-dimethyl ether (4), kaempferol-3,7,4'-trimethyl ether (5), dihydrobisdemethoxycurcumin (6) and bisdemethoxycurcumin (7).

### 2.3 Assay of antibacterial activity

The methodology to assess the antibacterial activity was followed that described previously (Leejae *et al.*, 2013). A stock solution (10 mg/ml) of the sample was prepared in dimethyl sulfoxide (DMSO) and diluted 2-fold to concentrations ranging from 0.5-256 µg/ml before experiment. The microorganisms, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922 were maintained in Tryptic soy broth (TSB) containing 20% glycerol at -80°C until required to use in the experiments. Each 100 µl of precultured microorganisms on Mueller-Hinton agar (MHA) at 37°C for 18 h were inoculated into 3 ml of Mueller-Hinton broth (MHB) separately and then further incubated at 37°C until mid exponential growth phase was obtained. The bacterial suspensions were diluted with the modified broth microdilution method as described by the Clinical and Laboratory Standards Institute (2011). Briefly, the microorganism suspensions were adjusted to the standard of McFarland number 0.5 with normal saline solution (NSS) to achieve a concentration of approximately 1.5×10<sup>8</sup> colony forming units per milliliter (CFU/ml). One hundred microliters of microorganism suspension was inoculated in 80 µl of MHB supplemented with 20 µl of the samples in each well of microtiter plates and then incubated at 37°C for 16-18 h. After that, the aliquots from the broth with no growth were spread onto fresh MHA plates using a sterile loop and incubated at 37°C for overnight. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the bioactive compounds were recorded in triplicate experiments.

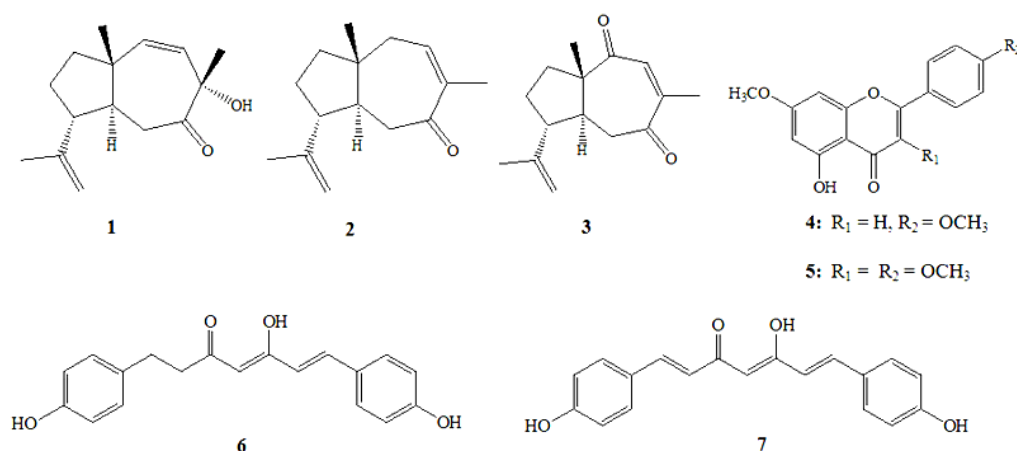


Figure 1. Chemical structures of 1-7 isolated from the rhizomes of *Bosenbergia kingii*

Vancomycin and 1% DMSO were used as positive and negative control, respectively.

## 2.4 Assay of HIV-1 IN inhibitory activity

**Oligonucleotide substrates:** Oligonucleotides of the long terminal repeat donor DNA (LTRD) and the target substrate (TS) DNA were bought from QIAGEN Operon, USA and stored at -25°C before use. The sequence of biotinylated LTRD and its unlabelled complement were 5'-biotin-ACCC TTTTAGTCAGTGTGGAAAATCTCTA GCAGT-3' (LTR-D1) and 3'-GAAAATCAGTCACACCTTTTAGAGATCGTCA-5' (LTR-D2), respectively; whereas those of the TS-DNA (digoxigenin-labelled target DNA, TS-1) and its 3-labelled complement were 5'-TGACCAAGGGCTAATTCAC-digoxigenin and digoxigenin-ACTGGTCCCCGATTAAGTGA-5' (TS-2), respectively.

**Multiplate integration assay (MIA):** The integration reaction was evaluated according to the method previously described (Tewtrakul *et al.*, 2006). Briefly, a mixture (45 µl) composed of 12 µl of IN buffer [containing 150 mM 3-(*N*-morpholino) propane sulfonic acid, pH 7.2 (MOPS), 75 mM MnCl<sub>2</sub>, 5 mM dithiothritol (DTT), 25% glycerol and 500 µg/ml bovine serum albumin], 1 µl of 5 pmol/ml digoxigenin-labelled target DNA and 32 µl of sterilized water were added into each well of a 96-well plate. Subsequently, 6 µl of sample solution and 9 µl of 1/5 dilution of integrase enzyme were added to the plate and incubated at 37°C for 80 min. After wells were washed with PBS four times, 100 µl of 500 mU/ml alkaline phosphatase (AP) labelled anti-digoxigenin antibody were added and incubated at 37°C for 1 h. The plate was washed again with washing buffer containing 0.05% Tween 20 in PBS three times and with PBS four times. Then, AP buffer (150 µl) containing 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM MgCl<sub>2</sub> and 10 mM *p*-nitrophenyl phosphate was added to each well and incubated at 37°C for 1 h. Finally, the plate was measured with a microplate reader at a wavelength of 405 nm. A control was composed of a reaction mixture, 50% DMSO and an integrase enzyme, while a blank was buffer-E containing 20 mM MOPS (pH 7.2), 400 mM potassium glutamate, 1 mM ethylenediaminetetraacetate disodium salt (EDTA·2Na),

0.1% Nonidet-P 40 (NP-40), 20% glycerol, 1 mM DTT and 4 M urea without the integrase enzyme. Suramin, a polyanionic HIV-1 IN inhibitor was used as a positive control.

$$\begin{aligned} & \% \text{ Inhibition against HIV-1 IN} \\ & = \left[ \frac{(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})}{\text{OD}_{\text{control}}} \right] \times 100 \end{aligned}$$

where OD is the absorbance detected from each well. The results of anti-HIV-1 IN activity were expressed as mean ± S.D. of four determinations. The IC<sub>50</sub> values were calculated using the Microsoft Excel program.

## 3. Results and Discussion

In this study, we examined the antibacterial and anti-HIV-1 IN activities of *B. kingii* extracts and its fractions as well as the isolated compounds for the alternative treatment of HIV/AIDS opportunistic infection using *in vitro* methods. The appearance of microbial resistance of many antibiotics and the occurrence of fatal opportunistic infections is one of the principal determinants of the morbidity and mortality associated with advanced HIV infection (Makhafola *et al.*, 2012; UNAIDS, 2014). The search for biologically active compounds based on traditionally use is still relevant. Therefore, *B. kingii* was evaluated for antibacterial, anti-HIV-1 IN activities. The ethanolic extract and its fractions of *B. kingii* were evaluated for antibacterial activity at the screening assay concentration (250 µg/ml) against both Gram-negative (*E. coli* ATCC 25922) and Gram-positive bacteria (*S. aureus* ATCC 29213) using the microdilution technique. The results of the ethanolic extract and its fractions are shown in Table 1. The ethanolic extract showed moderate antibacterial activity, while good activity was observed in chloroform fraction against *S. aureus* with MIC/MBC values of 32/128 and 16/32 µg/ml, respectively. However, *E. coli* was not inhibited by the ethanolic extract and its fractions at concentrations ≤ 250 µg/ml. As a result of this study, the active chloroform fraction was then separated by chromatographic method to yield seven compounds. All of the isolated compounds from the chloroform fraction showed antibacterial activity against Gram-positive bacteria at the concentration of 256 µg/ml.

Table 1. Antibacterial and anti HIV-1 IN activities of ethanolic extract and its fractions from *Bosenbergia kingii*

Sample	% Inhibition against HIV-1 IN at various concentrations (µg/ml)				MIC/MBC (µg/ml)	
	10	30	100	IC <sub>50</sub> (mg/ml)	<i>S. aureus</i>	<i>E. coli</i>
Ethanolic extract	-	-	-0.05 ± 6.99	> 100	32/128	> 256/NA
Hexane fraction	-	-	3.77 ± 7.60	> 100	128/> 256	> 256/NA
CHCl <sub>3</sub> fraction	8.52 ± 5.76	14.05 ± 0.18	68.56 ± 0.60	65.37	16/32	> 256/NA
EtOAc fraction	-	-	29.93 ± 5.77	> 100	256/> 256	> 256/NA
H <sub>2</sub> O fraction	27.77 ± 5.76	35.98 ± 2.85	84.48 ± 3.90	31.82	> 256/NA	> 256/NA
Vancomycin	-	-	-	-	0.5/1	-

Each value represents mean ± SD (N=4). (-) = not determined. NA = not applicable.

Table 2. Antibacterial and anti HIV-1 IN activities of isolated compounds from *Bosenbergia kingie*

Sample	% Inhibition against HIV-1 IN at various concentrations ( $\mu\text{M}$ )					IC <sub>50</sub> ( $\mu\text{M}$ )	MIC/MBC ( $\mu\text{g/ml}$ )	
	1	3	10	30	100		<i>S. aureus</i>	<i>E. coli</i>
Longiferone A (1)	-	-	-	-	-21.2 $\pm$ 0.6	>100	128/256	256/>256
Longiferone B (2)	-	-	-	-	-38.1 $\pm$ 6.3	>100	128/256	256/>256
Longiferone C (3)	-	-	-	-	-6.5 $\pm$ 1.5	>100	256/>256	256/>256
Kaempferol-7, 4'-dimethyl ether (4)	-	-	-	-	50.8 $\pm$ 1.6	>100	64/>256	>256/NA
Kaempferol-3,7,4'-trimethyl ether (5)	-	-	-	-	47.6 $\pm$ 3.3	>100	256/>256	>256/NA
Dihydrobisdemethoxycurcumin (6)	-	-	-	-	46.2 $\pm$ 1.4	>100	32/32	>256/NA
Bisdemethoxycurcumin (7)	-	-	23.5 $\pm$ 3.3	51.3 $\pm$ 4.3	56.8 $\pm$ 2.76	47.7	64/>256	>256/NA
Suramin	34.9 $\pm$ 1.9	45.7 $\pm$ 1.9	72.1 $\pm$ 2.1	85.3 $\pm$ 1.8	93.4 $\pm$ 1.66	2.9	-	-
Vancomycin	-	-	-	-	-	-	0.5/1	-

Each value represents mean  $\pm$  SD (N=4). (-) = not determined. NA = not applicable.

Compounds **1-3**, the sesquiterpenes, showed moderate inhibitory activity against *S. aureus* with MIC/MBC values of 128/256, 128/256 and 256/>256  $\mu\text{g/ml}$ , respectively (Table 2). They also possessed antibacterial activity against *E. coli* with MIC/MBC values of 256/>256, 256/>256 and 256/>256  $\mu\text{g/ml}$ , respectively. In addition, all three sesquiterpenes of *B. kingii* have been reported to show anti-inflammatory activity (Sudsai *et al.*, 2014) and this is the first scientific report on their antibacterial activity.

In addition, the presence of sesquiterpene daucane derivatives have been reported to have antibacterial together with antifungal activities in *Duncus carota* (Ahmed *et al.*, 2005). Thus, the sesquiterpene constituents isolated from *B. kingii* may play an essential role in the inhibition both bacteria strains. Compounds **4-5**, the flavonoids identified in chloroform fraction, showed a moderate inhibition only of Gram-positive bacteria strain with MIC/MBC of 64/>256  $\mu\text{g/ml}$  and 256/>256  $\mu\text{g/ml}$ , respectively. Moreover, good to moderate antimicrobial activities were observed in compounds **6** and **7** against *S. aureus* with MIC/MBC of 32/32  $\mu\text{g/ml}$  and 64/>256  $\mu\text{g/ml}$ , respectively. Compound **6** showed the most highest antibacterial activity. This is the first report of **6** on antibacterial effect, and it has also been reported to show antiplatelet and anti-inflammatory activities (Sudsai *et al.*, 2014, Dong and Chen *et al.*, 1998). In this study, *S. aureus* appeared to be more susceptible to the inhibitory effect of the compounds than *E. coli*. The weak activity of isolated compounds against *E. coli* was not surprising due to the differences in cell wall structure between Gram-positive and Gram-negative bacteria which led it be more resistant than Gram-positive ones. The Gram-negative bacteria have an outer membrane acting as a barrier to many environmental substances which greatly reduces the penetration of antibiotics through the cell wall (Pessini *et al.*, 2003). However, sesquiterpenes which are present in chloroform fraction may also contribute to the antibacterial activity might be affect on

permeability barriers of outer membrane of Gram-negative bacteria. Further studies in order to evaluate the mechanisms of action of these compounds as well as the antimicrobial activity against other microorganisms are needed.

Natural HIV-1 IN inhibitors have been reported from a variety of plants used in traditional medicines for the treatment of HIV/AIDS infected patients in Thailand such as *Eclipta prostrata*, *Piper betle*, *Spilanthus acmella*, *Zingiber zerumbet*, *Alpinia galanga* and *Boesenbergia pandurata* (Tewtrakul *et al.*, 2006). The ethanolic extract of *B. kingii* rhizomes exhibited weak anti-HIV-1 IN effect with an IC<sub>50</sub> of >100  $\mu\text{g/ml}$ . However, after partition with various organic solvents, the chloroform and aqueous fractions demonstrated HIV-1 IN inhibitory activity with IC<sub>50</sub> of 65.4 and 31.8  $\mu\text{g/ml}$ , respectively (Table 1). Although all the three compounds of sesquiterpenes (**1-3**) showed antibacterial activity, there were no positive results on their anti-HIV-1 IN activity (IC<sub>50</sub>>100  $\mu\text{M}$ ). The anti-HIV-1 IN activity of compounds **4** and **5**, flavonoids, were reduced by 50.8% and 47.6% at a concentrations of 100  $\mu\text{M}$ . Some flavonoides exhibit anti-HIV-1 effect *in vitro* and often have more than one mode of action. They can interact at different steps in the life cycle of HIV-1, including viral entry, reverse transcriptase, integrase, and viral protease (Zhang *et al.*, 2005). The action of **4** and **5** may be related to their inhibition on HIV-1 IN. Diarylheptanoids isolated from *B. kingii* (**6-7**) also exhibited anti HIV-1 IN activity with 46.2% and 56.8% at concentrations of 100  $\mu\text{M}$ , respectively. Compound **7** exhibited a moderate anti-HIV-1 IN with an IC<sub>50</sub> of 47.7  $\mu\text{M}$ . There are many scientific reports of diarylheptanoids on their anti-HIV-1 IN activity. Curcumin, a potent and safe compound has been identified as inhibitor of HIV-1 long terminal repeat-directed gene expression and block HIV replication by inhibiting HIV-1 IN, HIV-1 and HIV-2 protease (Itokawa *et al.*, 2008; Moghadamtousi *et al.*, 2014). Thus, the HIV-1 IN inhibitory activity of chloroform fraction of *B. kingii* may be due to the presence of phenolic com-

pounds, especially diarylheptanoid and flavonoid constituents, that may be related to one or more of these activities. In addition, phytochemical analysis of the most active inhibiting HIV-1 IN aqueous fraction of *B. kingii* was not studied as it was not found to possess antimicrobial activity in the present study. Therefore, further investigation on the isolation of active anti-HIV-1 IN activity constituents from the aqueous fraction of *B. kingii* might be of interest. The rhizomes of *B. kingii* used in traditional medicine possess various biological activities such as antioxidative, anti-inflammatory, and wound healing activities (Sudsai *et al.*, 2013; Sudsai *et al.*, 2014).

#### 4. Conclusions

This is the first report on the antibacterial and anti-HIV-1 IN activities of the ethanolic extract of *B. kingii* and its fractions. Moreover, the results of phytochemical study indicated that the sesquiterpenes, flavonoids and diarylheptanoids from this plant could possess antibacterial and anti HIV-1 IN effects. Thus, these studies may support the traditional uses of the *B. kingii* for treatment of HIV/AIDS opportunistic diseases and provide the basic data for further investigation to isolate other active constituents.

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