

CONSTITUENTS AND INHIBITORY EFFECT ON HUMAN PATHOGENIC BACTERIA OF THE ROOTS OF *SCUTELLARIA BAICALENSIS*[#]

Le Duc Long¹, Nguyen Huu Tung², Nguyen Thi Thom³, Gyung Ja Choi⁴,
Vu Dinh Hoang³, Le Dang Quang^{1,*}

¹R&D Center of Bioactive Compounds, Vietnam Institute of Industrial Chemistry,
22 Pham Ngu Lao, Hoan Kiem, Ha Noi

²VNU School of Medicine and Pharmacy, Vietnam National University, 144 Xuan Thuy,
Cau Giay, Ha Noi

³Hanoi University of Science and Technology, 1 Dai Co Viet, Hai Ba Trung, Ha Noi

⁴Center for Eco-friendly New Materials, Korea Research Institute of Chemical Technology,
141 Gajeong-Ro, Yuseong-Gu, Daejeon, 305-600, Republic of Korea

*Email: ledangquang2011@gmail.com

Received: 23 July 2018; Accepted for publication: 15 November 2018

Abstract. Methanol extract of the roots of *Scutellaria baicalensis* effectively inhibited the bacterial growth of human pathogenic bacteria *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 21768 and *Bacillus subtilis* ATCC 6633 at MICs of 2,000 µg/mL. Hexane, ethyl acetate and aqueous residues were prepared by successively partitioning the methanol extract with hexane and ethyl acetate. Among them, only ethyl acetate layer showed antibiotic effect; whereas hexane and aqueous layers were inactive against tested bacteria. The ethyl acetate residue was fractionated by silica gel column chromatography to afford three flavonoids and an oligosaccharide. Their chemical structures were elucidated as wogonin (**SB1**), baicalein (**SB4**), baicalin (**SB5**) and tetrasaccharide (**SB10**) on the basis of the analysis of NMR and MS spectroscopic data. The isolates were evaluated for *in vitro* inhibitory effect against human pathogenic bacteria using micro dilution bioassay method. Baicalein (**SB4**) showed a broad-spectrum inhibition against various human pathogenic bacteria. In particular, it was found to potently inhibit *S. aureus* ATCC 6538 and *B. cereus* ATCC 21768 with MICs of 9.5 and 38 µg/mL, respectively. The study results demonstrated antibiotic effect of the extracts from the roots of *S. baicalensis* and characterization of compounds isolated from the plant materials.

Keywords: activity, flavonoid, isolation, *Scutellaria baicalensis*.

Classification numbers: 1.2.1.

[#] Presented at the Conference: "The 6th National Workshop on Research and Development of Natural Products"

1. INTRODUCTION

The genus *Scutellaria* consists of over 350 species worldwide and has been used by many cultures to treat a variety of medical conditions, including anxiety, nervous disorders, liver disease and cancers [1]. *S. baicalensis* is widely grown in Vietnam where root decoction has traditionally been used in medicine as a cancer treatment, remedy for inflammation and atherosclerosis [1, 2]. The flavonoids such as baicalein, wogonin, and baicalin are the major compounds in *S. baicalensis*. In recent years, the flavonoids isolated from the roots of *S. baicalensis* have demonstrated therapeutic potential for anti-inflammatory, anti-HIV, anti-human herpes virus type 6 (HHV-6), antioxidant, neuroprotective and anti-cancer properties [3]. The antibiotic activity of the roots of *S. baicalensis* and its constituents are also considered because this plant has a wide range of uses in traditional medicine. The aim of this study, therefore, is to isolate and identify metabolites from the roots of *S. baicalensis* and to test for their antimicrobial activity against the growth of human pathogenic bacteria.

2. MATERIALS AND METHODS

2.1. Bacterial strains and culture conditions

The extracts and the isolated compounds were tested for their antibacterial activity against Gram-positive bacteria such as *Bacillus cereus* ATCC 21768, *Bacillus subtilis* ATCC 6633, and *Staphylococcus aureus* ATCC 6538 which were obtained from the ATCC (Manassas, VA, USA) and Gram-negative bacteria such as *Escherichia coli* (American Type Culture Collection) ATCC 25922, *Burkholderia cepacia* ATCC 25416, *Enterobacter cloacae* ATCC 13047 and *Pseudomonas aeruginosa* ATCC 9027. The bacterial strains were activated on nutrient agar (NA), then, transferred in nutrient broth (NB) at 37 °C for 24 h. The bacterial suspensions were diluted with sterile saline to get a turbidity equivalent to that of the 0.5 McFarland standard.

2.2. Plant materials

Scutellaria baicalensis Georgi was purchased in Ninh Hiep folk medicine market in August 2016 and identified by Mr. Nghiem Duc Trong from the Department of Botany, Hanoi University of Pharmacy. Materials were crushed to powdery sizes of from 0.1 to 3 mm. The root powder (moisture content 7-10 %) was kept in dry environment at R&D Center of Bioactive Compounds, Vietnam Institute of Industrial Chemistry prior to extraction experiments.

2.3. Isolation of constituents from *Scutellaria baicalensis*

The roots of *S. baicalensis* (2 kg) were crushed and extracted three times with methanol at 65°C for 8 h and then filtrated and evaporated under reduced pressure to yield 390 g crude methanol extract. Then, the crude methanol extract was dissolved with 1.5 L of distilled water and partitioned with hexane and ethyl acetate (1.5 L; 3 times for each), consecutively. Each organic layer was evaporated under reduced pressure to yield hexane soluble residue (41.5 g) and ethyl acetate soluble residue (237.6 g).

The hexane and ethyl acetate residue were tested by thin layer chromatography; the presence of flavonoids and oligosaccharides was observed in the ethyl acetate residue. The ethyl acetate residue (20 g) was subjected to column chromatography on silica gel (400 g silica gel 60Å (40–63 µm), 4.5 × 70 cm) which was eluted with the gradient solvent system of

hexane/ethyl acetate (10:0; 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, 0:10, v/v) and finally washed out with the mixture of ethyl acetate and methanol in the ratio of 8:2 (v/v). In the results 7 fractions F1-F7 were yielded. Fraction 2 was fractionated on a Sephadex LH-20 column (3 g, 2.0 × 70 cm) eluted with methanol and the selected fractions were crystallized with hexane to give crystals of **SB1** (842.7 mg). Fraction 4 (2.986 g) was applied on a silica gel column (60 g silica gel 60 Å (40–63 μm), 3.0 × 40 cm), eluted with hexane/ethyl acetate (7:3; 6:4, 5:5, v/v, 240 mL each and fraction volume of 50 mL) to yield two pure compounds **SB4** (46.7 mg) and **SB5** (387 mg). Fraction 6 (3 g) was dissolved with 150 mL of methanol and mixed with 4.5 g of silica gel 60 Å (40–63 μm). Then, the mixture was fractionated by a silica gel column chromatography (90 g silica gel 60Å (40–63 μm), 3.4 × 50 cm), eluted with a gradient system of ethyl acetate/methanol (8:2, 75:25, 7:3, 65:35, 6:4, 55:45, 5:5, 45:55, 4:6, 45:65, v:v) and then washed out with methanol:water in the ratio of 40:1 (v/v) to produce four fractions, F61 to F64. Fraction F61 was separated on a Sephadex LH-20 column (2 g, 2.0 × 50 cm) eluted with methanol to yield **SB10** (468 mg).

2.4. Structural characterization of the isolated compounds

SB1: Wogonin

ESI-MS m/z 284.07 [M+H]⁺; ¹H-NMR ((CD₃)₂CO, 500 MHz): 6.328 (1H, s, H-3), 6.817 (1H, s, H-6), 8.122 (1H, dd, $J=7$ Hz, $J=1.5$ Hz, H-2'), 7.642 (2H, m, $J=8.5$ Hz, H-3', H-5'), 7.646 (1H, m, H-4'), 8.122 (1H, dd, $J=5$ Hz, $J=3.5$ Hz, H-6'); ¹³C-NMR ((CD₃)₂CO, 125 MHz): 164.64 (C-2), 106.25 (C-3), 183.45 (C-4), 150.93 (C-5), 158.46 (C-8), 158.03 (C-7), 99.99 (C-6), 130.26 (C-9), 105.62 (C-10), 132.50 (C-1'), 127.80 (C-2', C-6'), 128.93 (C-3', C-5'), 132.96 (C-4').

SB4: Baicalein

ESI-MS m/z 293.2 [M+Na]⁺, m/z 271.2 [M+H]⁺; ¹H-NMR ((CD₃)₂CO, 500 MHz): 6.76 (1H, s, H-3), 12.76 (1H, s, 5-OH), 6.68 (1H, s, H-8), 8.07 (2H, dd, $J=1$ Hz, $J=7.5$ Hz, H-2', H-6'), 7.58 (2H, m, H-3', H-5'), 7.6 (1H, m, H-4'); ¹³C-NMR ((CD₃)₂CO-*d*₆, 125 MHz): 164.75 (C-2), 105.86 (C-3), 183.52 (C-4), 147.87 (C-5), 153.82 (C-6), 151.70 (C-7), 94.9 (C-8), 130.09 (C-9), 105.59 (C-10), 132.63 (C-1'), 127.31 (C-2', C-6'), 129.89 (C-3', C-5'), 132.69 (C-4').

SB5: Baicalin

ESI-MS m/z 447,1 [M+H]⁺; ¹H-NMR (DMSO-*d*₆, 500 MHz): 7.06 (1H, s, H-3), 7.01 (1H, s, H-8), 8.07 (2H, m, H-2', H-6'), 7.61 (2H, m, H-3'), 7.63 (1H, m, H-4'), 12.60 (1H, s, 5-OH), 8.69 (1H, s, 6-OH); ¹³C-NMR (DMSO-*d*₆, 125 MHz): 163.55 (C-2), 106.14 (C-3), 182.56 (C-4), 146.78 (C-5), 130.62 (C-6), 151.28 (C-7), 93.74 (C-8), 149.21 (C-9), 104.76 (C-10), 130.85 (C-1'), 126.39 (C-2', C-6'), 129.18 (C-3', C-5'), 132.06 (C-4'), 99.93 (C-1''), 72.81 (C-2''), 75.25 (C-3''), 71.33 (C-4''), 75.51 (C-5''), 170.08 (C-6'').

SB10: Tetrasaccharide (*O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-fructofuranosyl-(1→6)-*O*-α-D-glucopyranosyl-(1→2)-*O*-β-D-fructofuranoside).

ESI-MS m/z 689.1 [M+Na]⁺; ¹H-NMR (CD₃OD, 500 MHz): 5.156 (1H, s, H-1), 3.737 (1H, s, H-2), 3.799 (1H, t, H-3), 3.519 (1H, t, H-4), 4.626 (1H, t, H-5), 3.628 (1H, t, H-6), 3.705 (1H, s, H-7), 3.985 (1H, s, H-9), 3.767 (1H, s, H-10), 4.530 (1H, t, H-11), 3.417 (1H, m, H-12), 5.148 (1H, s, H-1'), 3.726 (1H, s, H-2'), 3.777 (1H, t, H-3'), 3.501 (1H, t, H-4'), 3.598 (1H, t, H-6'), 3.690 (1H, s, H-7'), 3.888 (1H, s, H-9'), 3.761 (1H, t, H-10'), 4.514 (1H, t, H-11'), 3.410 (1H, m, H-12'); ¹³C-NMR (CD₃OD, 125 MHz): 93.80 (C-1), 72.87 (C-2), 76.59 (C-3), 69.26 (C-4), 77.88 (C-5), 62.69 (C-6), 65.76 (C-7), 99.15 (C-8), 77.85 (C-9), 76.59 (C-10), 83.03 (C-11),

62.52 (C-12), 98.00 (C-1'), 71.53 (C-2'), 76.11 (C-3'), 71.09 (C-4'), 77.81 (C-5'), 64.45 (C-6'), 64.97 (C-7'), 103.05 (C-8'), 77.36 (C-9'), 76.11 (C-10'), 83.03 (C-11'), 62.55 (C-12').

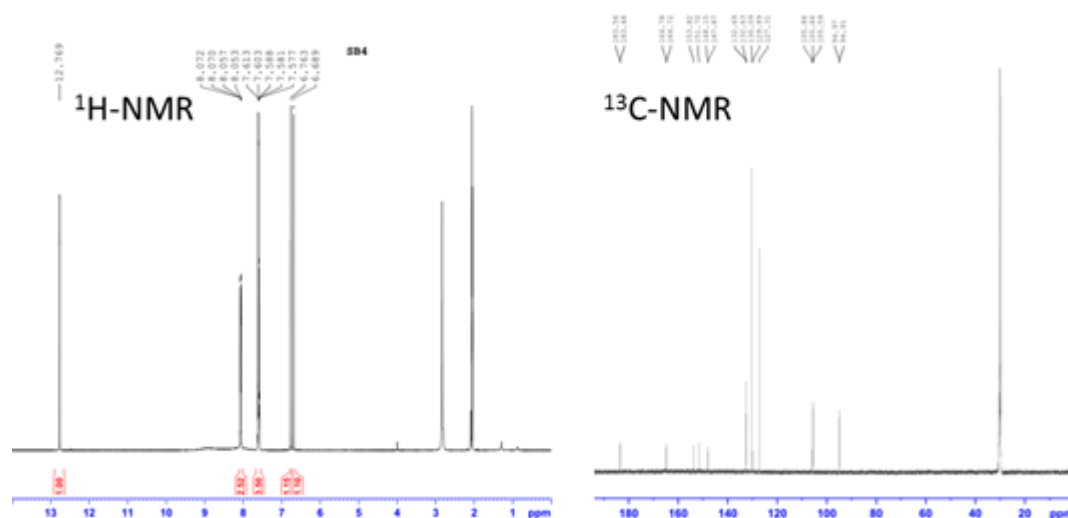


Figure 1. The ^1H -NMR and ^{13}C -NMR spectra of baicalein (SB4). NMR spectra were recorded of an acetone- d_6 solution of SB4 on a Bruker AMX-500 (500 MHz). Chemical shifts are given in parts per million (ppm) downfield from tetramethylsilane.

2.5. Instrumental analyses

Nuclear magnetic resonance (NMR) data of the isolated compounds were recorded on a Bruker AMX-500 (500 MHz) spectrometer (Bruker Analytische Messtechnik GmbH, Rheinstetten, Germany) at 500 MHz for ^1H -NMR spectra and 125 MHz for ^{13}C -NMR spectra (Fig. 1). Tetramethylsilane (TMS) is using as an internal standard. The electrospray ionization mass spectra (ESI-MS) of the isolated compounds were recorded on an MSD1100 single-quadrupole mass spectrometer equipped with an electrospray ionizer (Hewlett-Packard Co., Palo Alto, CA, USA).

Chemical structures of the isolates were determined by NMR data analyses and comparison with those of the previously reported in literature.

2.6. *In vitro* antibacterial assay against human pathogenic bacteria

All bacterial strains were grown on nutrient agar (NA) and nutrient broth (NB) at 37°C for 24 h and then suspended in sterile saline at a density equivalent to that of the 0.5 McFarland standards. Bacterial suspensions with a concentration of 10^5 cfu/mL were used for *in vitro* antibacterial activity test.

In vitro antibacterial assay was conducted in 96-well microtiter plates on the basis of a modification of broth micro-dilution method [4]. The tested materials were dissolved in dimethyl sulfoxide (DMSO) to get the stock solutions with dilution factor of 100. The working concentrations of the extracts and isolated compounds ranged from 9.5 to 2,000 $\mu\text{g}/\text{ml}$. DMSO (2 %) was used as the negative control, at which it did not affect the bacterial growth. Chloramphenicol (Sigma-Aldrich, USA) was used as a positive control against all the bacteria. The assay was repeated twice with two replicates for each sample against the individual bacterial species at all the test concentrations. The minimum inhibitory concentration (MIC, $\mu\text{g}/\text{mL}$) was

determined as the lowest concentration that completely inhibited the growth of the bacteria which were incubated at 37°C for 1 day and 2 days. The optical density (OD) was measured spectro-photometrically at 600 nm. The growth inhibition for each dilution was determined using the formula:

$$\text{Percent inhibition (\%)} = 100 \times [1 - \text{OD of treated well} / \text{OD of negative control well}],$$

where OD is the optical density of each well 24h after incubation; values of OD of negative control well and OD of treated well were corrected with OD of its blank wells corresponding to each concentration.

3. RESULTS AND DISCUSSION

3.1. Isolation and characterization of metabolites from the roots of *S. baicalensis*

Compounds **SB1**, **SB4** and **SB5** were detected to be flavonoids which produced dark green color with FeCl₃ and yellow color with KOH/ethanol reagents. Their ¹³C-NMR spectra data displayed that all of the compounds have 15 carbons in flavonoid skeleton which consists of two phenyl rings (A and B-rings) and heterocyclic ring (C-ring). In the A-ring of each structure, a carbonyl group at C-4 was presented in a range of δ 183.45-183.56 ppm; two oxygenated aromatic carbons at C-5 and C-7 in **SB1** did from δ 150.62 to 158.03 ppm. The heterocyclic ring revealed the presence of phenolic group in a range of δ 7.06-8.69 ppm in ¹H-NMR spectra of the three compounds. The ¹H-NMR spectrum of **SB1** displayed a signal at δ 6.328 ppm that is expected for an olefinic proton and appeared as a singlet. A signal δ 6.817 ppm also appeared as a singlet, which is assigned as a proton at C-6 (δ 99.99 ppm) in the A-ring. Two sets of protons in the B-ring (H-2', H-6' and H-3', H-5') are symmetrical in each pair. Its ¹³C-NMR spectral data showed that the δ 158.46 ppm represented at C-8 position of A-ring connected to a methoxy group. By the ¹H-NMR and ¹³C-NMR spectra data, **SB1** was determined to be wogonin by comparison with NMR spectral data from previously published literatures [5]. **SB4** was deduced to be baicalein based on the analysis of ¹H- and ¹³C-NMR spectra (Figure 1). In the structure of baicalein, aromatic oxygenated carbons δ 147.87 (C-5), 153.82 (C-6) and 151.70 (C-7) in A-ring are connected to hydroxyl groups may be corresponding in antibacterial activity (Figure 2) [6].

Structure of compound **SB5** is the similar to that of compound **SB1**. The ¹³C-NMR spectrum of **SB5** showed 19 signals arising from one flavone skeleton including 15 carbons (C-2 to C-10 and C-1' to C-6'). ¹H-NMR and ¹³C-NMR spectra of **SB5** revealed a hexanoic pattern having an acid group (δ 71.33, 72.81, 75.25, 75.51, 99.93, 170.08 ppm), whose chemical shift values were in good agreement with those of β-D-glucuronic acid. Based on symmetry considerations, two carbon atoms of the B-ring were chemically equivalent; hence, only 13 signals from the 15 carbons were observed. Instead of a hexose, a hexanoic acid was present: the strongly deshielded resonance from the carbon atom at δ 170.08 ppm is the result of an acid functional group. The NMR data obtained for compound **SB5** are consistent with that of baicalin [7].

Compound **SB10** was obtained as white colorless crystals. The ¹³C-NMR and JMOD spectra showed twenty-four carbons in the range from δ 62.55 to 103.05 ppm, which is characteristic of a tetrasaccharide corresponding to the carbons bonded with oxygen at position of those in glucose and fructose. Its ¹H-NMR spectrum showed anomeric proton signals and symmetry in pairs. Two signals at δ 5.156 (1H, s, H-1) and 5.148 (1H, s, H-1') were determined to be two protons anomeric in two glucose moieties. Two signals of anomeric carbons at 93.80 and 98.00 ppm together with two quaternary oxygenate carbons at δ 99.15 and 103.05 suggested

that this compound comprised two glucose molecules and two fructose molecules. Compared with the previously reported data in the literature, compound **SB10** was identified as *O*- α -D-glucopyranosyl-(1 \rightarrow 2)-*O*- β -D-fructofuranosyl-(1 \rightarrow 6)-*O*- α -D-glucopyranosyl-(1 \rightarrow 2)-*O*- β -D-fructofuranoside (Figure 3), an analog of sucrosyl-(1 \rightarrow 2)- β - isomaltulose [8].

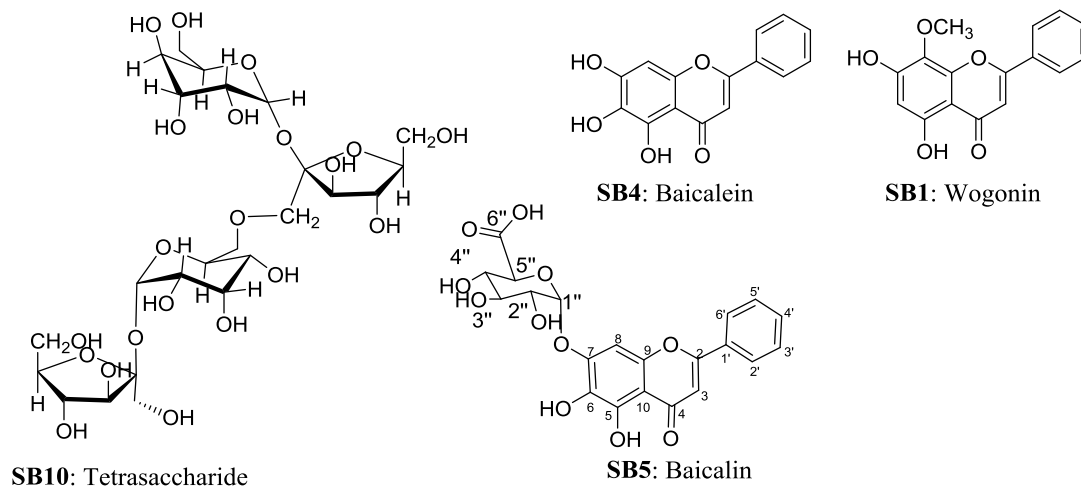


Figure 2. Chemical structure of compounds isolated from the roots of *S. baicalensis*.

3.2. Antibacterial activity of layers and isolated compounds from the roots of *S. baicalensis*

In the study, the methanol extract and the two compounds as **SB1** and **SB4** were selected for testing against seven human pathogenic bacteria. MICs values ($\mu\text{g/mL}$) at 1 day and 2 days after incubation were detected by visualizing with naked eye and listed in Table 1. **SB1** was not active against all bacterial strains at the range of tested concentrations. Methanol extract of *S. baicalensis* inhibited the bacterial growth of *S. aureus* ATCC 6538, *B. cereus* ATCC 21768 and *B. subtilis* ATCC 6633 with the same MICs of 2,000 $\mu\text{g/mL}$. **SB4** was found to potently inhibit bacterial strains such as *S. aureus* ATCC 6538 and *B. cereus* ATCC 21768 with MICs of 9.5 and 38 $\mu\text{g/mL}$, respectively.

Table 1. MIC values of the methanol extract and isolated compounds from *S. baicalensis* against human pathogenic bacterial strains.

| Bacterial strains | MIC ($\mu\text{g/mL}$) ^a | | | |
|-------------------------------|---------------------------------------|------------------------------------|------------------|------------|
| | SB1 | SB4 | Methanol extract | Chl |
| <i>S. aureus</i> ATCC 6538 | - | 9.5 ^b (19) ^c | 2,000 (2,000) | 40 (40) |
| <i>B. cereus</i> ATCC 21768 | - | 38 (75) | 2,000 (2,000) | 40 (40) |
| <i>B. subtilis</i> ATCC 21768 | - | 1,000 (2,000) | 2,000 (2,000) | 40 (40) |
| <i>B. cepacia</i> ATCC 25416 | - | 1,000 (>2,000) | - | 20 (20) |

^aMIC: minimum inhibition concentration ($\mu\text{g/mL}$) is the lowest concentration of antimicrobial agent that completely inhibits growth of the organism in microdilution wells as detected by the naked eye. ^bMIC values at 1 day after incubation. ^c(MIC) values at 2 days after incubation. (-) no inhibition. **SB1**: wogonin; **SB4**: baicalein. **Chl**: chloramphenicol.

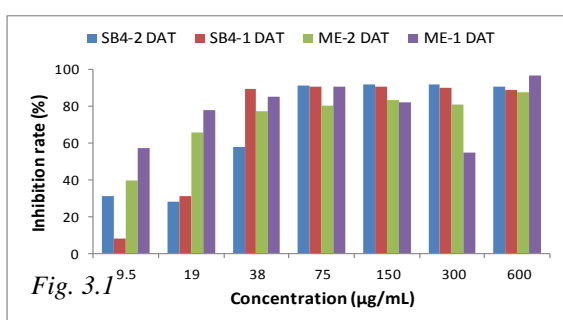


Fig. 3.1

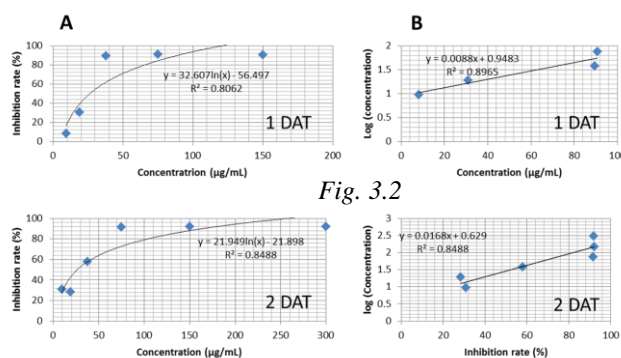


Fig. 3.2

Figure 3. Fig 3.1: Effects of baicalein (SB4) and methanol extract (ME) of *Scutellaria baicalensis* on the bacterial growth of *Bacillus cereus* ATCC 21768 at 1 day (1 DAT) and 2 days (2 DAT) after incubation.

Fig. 3.2. *In vitro* dose-response of baicalein for antibacterial activity against *B. cereus* ATCC 21768. (A) Dose-effect and (B) Log of dose-effect curves.

Table 2. Calculated inhibitory concentrations IC₅₀ and IC₉₀ of baicalein causing 50 % and 90 % inhibition against the bacterial growth of *Bacillus cereus* ATCC 21768.

| Conc. (µg/mL) | 1 DAT | 2 DAT |
|------------------|---|---------------------|
| IC ₅₀ | 25.8 ^a (11.0- 60.5) ^b | 32.7 (5.6 - 56.8) |
| IC ₉₀ | 78.6 (40.4-152.9) | 109.7 (55.3 -164.0) |

^aInhibition values were calculated on the basis of Probit analysis of dose-response data expressed in Fig. 3.2. ^b 95 % confidence interval.

Besides, it significantly affected the bacterial growth of *B. subtilis* ATCC 6633 and *B. cepacia* ATCC 25416 strains at 1,000 µg/mL. The effects of **SB4** and methanol extract on the bacterial growth of *B. cereus* ATCC 21768 were further tested by broth micro-dilution method in 96-well plates and their inhibition rates were presented in Figure 3.

Baicalein (**SB4**) showed a dose-response activity against *B. cereus* ATCC 21768 (Figure 3.2); it effectively inhibited the bacterial growth with low IC₅₀ and IC₉₀ values in a range of 25.8 to 109.7 µg/mL (Table 2). The difference between the antibacterial efficacy of **SB1** and **SB4** against *B. cereus* ATCC 21768 may be due to a different pattern of hydroxyl and methoxy groups at positions C-5, C-6, C-7 and C-8 in A-ring of each compound. Three bacterial strains *E. coli* ATCC 25922, *P. aeruginosa* ATCC 9027 and *E. cloacae* ATCC 13047 were resistant to all of the test materials (data not shown).

In addition, our results of the potent antibacterial activity against *B. cereus* ATCC 21768, notably, the inhibition of baicalein against the bacterial growth of *S. aureus* ATCC 6538 is highly paid attention by natural product chemists and be evident by certain studies in synergistic effects of baicalein with other antibiotics such as ciprofloxacin, oxacillin and vancomycin [10].

4. CONCLUSIONS

The present study exhibited that methanol extract of *S. baicalensis* had an inhibition for bacterial growth of *S. aureus* ATCC 6538, *B. cereus* ATCC 21768 and *B. subtilis* ATCC 6633.

Its major constituents were identified as wogonin (**SB1**), baicalein (**SB4**), baicalin (**SB5**) and a tetrasaccharide (**SB10**). Of them, **SB4** strongly inhibited bacterial strains such as *S. aureus* ATCC 6538, *B. cereus* ATCC 21768 and *B. subtilis* ATCC 6633. Our study suggested that *S. baicalensis* is a good plant resource containing potent antibacterial substances for developing botanical drugs to treat human bacterial pathogens.

Acknowledgements. The research was financially supported by the project of MOIT (Code: 049.17.ĐT/HĐ-KHCN).

REFERENCES

1. Konoshima T., Kokumai M., Kozuka M., Iinuma M., Mizuno M., Tanaka T., Tokuda H., Nishino H., Iwashima A. - Studies on inhibitors of skin tumour promotion. XI. Inhibitory effects of flavonoids from *Scutellaria baicalensis* on epstein-barr virus activation and their anti-tumour-promoting activities, *Chem. Pharm. Bull.* **40** (2) (1992) 531-533.
2. Guo Q., Zhao L., You Q., Yang Y., Gu H., Song G., Lu N., Xin J. - Anti-hepatitis B virus activity of wogonin *in vitro* and *in vivo*, *Antiviral Res.* **74** (2016) 16-24.
3. Chen X., Zhang C. - Inhibitory role of baicalin on human herpes virus type 6 *in vitro*, *Rocedia Engineering* **37** (2012) 75-78.
4. Vu T. T., Kim H., Vu T. K., Le Dang Q., Nguyen H.T., Kim H., In K. S., Choi J. G., Kim J.-C. - *In vitro* antibacterial activity of selected medicinal plants traditionally used in Vietnam against human pathogenic bacteria, *BMC Complement Altern. Med.* **16** (32) (2016) PMID 26819218.
5. Delange D. M., Rico C. L. M., Canavaciolo V. G., Cuellar A. C., Oliver E. S. - Selective and high yield isolation of pure wogonin from aerial part of *Scutellaria havanensis* Jacq., *Int. J. Pharm. Sci. Rev. Res.* **30** (2015) 104-108.
6. Pegg R. B., Amarowicz R., Oszmianski J. - Confirming the chemical structure of antioxidativetri hydroxyflavones from *Scutellaria baicalensis* using modern spectroscopic methods, *Pol. J. Food Nutr. Sci.* **14** (2005) 43-50.
7. Zhou Y., Hirotani M., Yoshikawa T., Furuya T. - Flavonoids and phenylethanoids from hairy root cultures of *Scutellaria baicalensis*, *Phytochem.* **44** (1997) 83-87.
8. Ehrhardt S, Rittig F, Vogel M, Wray V, Skeries B. - Sucrosyl-(1 → 2)-β-isomaltulose: enzymatic synthesis and structure determination. *J. Carbohyd. Chem.* **23** (2–3) (2004) 163-168.
9. Chan B. C., Ip M., Lau C. B., Lui S. L., Jolivald C., Ganem-Elbaz C., Litaudon M., Reiner N. E., Gong H., See R. H., Fung K. P., Leung P. C. - Synergistic effects of baicalein with ciprofloxacin against Nora over-expressed Methicillin-Resistant *Staphylococcus aureus* (MRSA) and inhibition of MRSA pyruvate kinase, *J. Ethnopharmacol.* **137** (2011) 767-773.
10. Cai W., Fu Y., Zhang W., Chen X., Zhao J., Song W., Li Y., Huang Y., Wu Z., Sun R., Dong C., Zhang F. - Synergistic effects of baicalein with cefotaxime against *Klebsiella pneumoniae* through inhibiting Ctx-M-1 gene expression, *BMC Microbiol.* **16** (2016) 181.