Antibacterial Activity of (2S)-7,4'-dihydroxy-5-methoxy-8-(γ, γ-dimethylally)-flavanone against Methicillin-Resistant *Staphylococcus aureus*

Eun Sook Kim*

Vestibulocochlear Research Center & Department of Microbiology, School of Medicine, Wonkwang University

The emergence of methicillin-resistant of *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) has led to an urgent need for the discovery and development of new antibacterial agents. As part of an ongoing investigation into the antibacterial properties of the natural products, (2S)-7,4'-dihydroxy-5-methoxy-8-(γ, γ -dimethylally)-flavanone (2S-DMDF), isolated from the roots of Sophora flavescens, was found to be antibacterial active MRSA and VRE. Sophora flavescens has been used as antibacterial, antiviral, antiprotozoal, anti-inflammatory. Therefore, this study investigated the antibacterial activity of 2S-DMDF against all the bacterial strains tested. In this result, at the end point of an optically clear well, the minimum inhibitory concentrations (MICs) ranged from 0.97 to 15.6 mg/ml for 2S-DMDF, from 125 to 256 mg/ml for ampicillin, and from 64 to 512 mg/ml for gentamicin with MRSA, also, 7.8 to 15.6 mg/ml for 2S-DMDF, from 125 to 256 mg/ml for ampicillin, and from 512 to 1024< mg/ml for vacomicin with VRE. These findings indicated that the application of the tested 2S-DMDF alone might prove useful in the control and treatment of MRSA and VRE infections.

Key words : Sophora flavescens, enterotoxin gene, (2S)-7,4'-dihydroxy-5-methoxy-8-(γ, γ-dimethylally)-flavanone, MRSA, VRE

Introduction

Gram-positive bacteria are common causes of nosocomial infections. Since an outbreak of vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) were reported, outbreaks of nosocomial VRE and MRSA infection have been increasingly reported worldwide.

The incidence of infectious disease caused by drug resistant bacteria that are represented by MRSA, VRE and so on is extremely important in both community and hospital circumstances[1]. Causes of this rising incidence include the inappropriate and excessive use of antibiotics and insufficient hygiene in the hospital environment[1,2]. Also, MRSA has been known to one of the most common pathogen of chronic otitis media (COM) and skin infection. It can cause not only middle ear infection but also other complications in inner ear and brain such as sensorineural hearing loss[3,4].

* To whom correspondence should be addressed at : Kim Eun Sook, Vestibulocochlear Research Center & Department of Microbiology, School of Medicine, Wonkwang University, 344-2, Shinyong-dong, Iksan, Jeonbuk · E-mail : bacteria-g@hanmail.net, · Tel :063-850-5678

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Many *Staphylococcus aureus* strains produce one or more of a group of specific enterotoxin that include staphylococcal enterotoxins (SEs), staphylococcal exfoliative toxins (ETs), and toxic shock syndrome toxin 1 (TSST-1). The SEs are cause staphylococcal food poisoning[5,6]. The ETs are responsible for the staphylococcal scalded-skin syndrome[7]. TSST-1 is the major exotoxin etiologically involved in staphylococcal toxic shock syndrome, especially in menstrual cases[8]. Multi-drug resistant and the infectious diseases caused by MRSA are intense problems[9]. Thus, we have been trying to discover effective growth inhibitors against MRSA and VRE. Sophorae Radix, the dried roots of Sophora flavescens, has been used in oriental traditional medicine for treatment of skin, mucosal, diarrhea, inflammation, abscess, dysentery, also, it has been well-known antibacterial, antiviral, antiprotozoal, anti-inflammatory. This plant is reported to harbor quinolizidine alkaloids, triterpenoid saponins, and prenylated flavonoids, which are responsible for various biological and pharmacological properties[10].

Here, we report new natural compounds, isolated from root of Sophora flavescens and antimicrobial activity of (2S)-7,4'-dihydroxy-5-methoxy-8-(γ, γ-dimethylally)-flavanone (2S-DMDF) against MRSA with enterotoxin gene of COM and

VRE with vanA gene.

Materials and Methods

1. Plant material

The roots of Sophora flvescens used in the present study was collected in October 2006 from Jinan, Province of Jeonbuk, South Korea. This plant was identified and authenticated by Prof. Hong Jun Kim at the College of Oriental Medicine, Woosuk University. A voucher specimen (No. JSI0903) has been deposited at deposited in the author's laboratory.

2. Extraction and isolation of (2S)-7,4′-dihydroxy-5-methoxy-8-(ɣ, ɣ-dimethylally)-flavanone

The shade dried roots of S. flavescens (3.2 kg) were crushed and extracted three times with MeOH under reflux. The MeOH extract was concentrated, suspended in H_2O and sequentially partitioned with $CHCl_3$, EtOAc and n-BuOH. The soluble $CHCl_3$ fraction (120 g) was subjected to silica gel column chromatography eluted with $CHCl_3$-EtOAc-MeOH (15:1:1) to afford nine fractions. Biological activity was concentrated in fraction 4(14 g), which was further purified on silica gel column chromatography with $CHCl_3$-EtOAc-MeOH (10:1:1) to yield four subfractions(fraction 1-4). The 2 fractions (2.7 g) underwent a final purification with preparative HPLC (JALGS column, MeOH, 3 mL/min and 210 nm) to yield compound 1 (37.4 mg). The structure of 2S-DMDF was identified by the spectral data (MS, 1D NMR and 2D NMR) with those reported in the literature.[11] The purity of (E)-2,3,5,4′-tetrahydroxystilbene-2-O-β-D-glucoside was proven by HPLC as > 98%. 2S-DMDF: 1H-NMR (500MHz, DMSO) δ 1.53 (3H, s, H-5″), 1.57 (3H, s, H-4″), 2.87 (2H, dd, J = 16.5, 6.9Hz, H-3), 3.10 (2H, d, J = 7.0Hz, H-1″), 5.10 (1H, brt, J = 7.0Hz, H-2″), 5.28 (1H, dd, J = 12.4, 3.0Hz, H-2), 6.12 (H, s, H-6), 6.77 (2H, d, J = 8.4Hz, H-3′ and H-5′), 7.27 (2H, d, J = 8.4Hz, H-2′ and H-6′). 13C-NMR(125MHz, DMSO) δ 187.22 (C-4), 163.10 (C-7), 161.21 (C-9), 159.70 (C-5), 157.32 (C-4′), 129.75 (C-1′), 129.49 (C-3″), 127.69 (C-2′and C-6′), 123.29 (C-2″), 115.00 (C-3′ and C-5′), 107.53 (C-8), 103.51 (C-10), 93.25 (C-6), 77.67 (C-2), 55.16 (C5-OCH3), 44.73 (C-3), 25.48 (C-5″), 21.57 (C-1″), 17.54 (C-4″).

3. Preparation of bacterial strains

A total of 5 MRSA with enterotoxin gene and 5 VRE with vanA gene strains were clinical isolates from Wonkwang University Hospital (Iksan, Korea) and the standard strain of Staphylococcus aureus ATCC 33591 and VRE 24 (vanA). The MRSA strains were defined on the basis of the occurrence of the mecA gene and of their resistance to ampicillin and oxacillin, VRE strains were defined expression of the vanA gene and of their resistance to vancomycin according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2009)[12]. The MRSA and VRE strains were defined after culturing all strains on Mueller-Hinton agar (Difco, Detroit, MI), all bacteria were resuspended in Mueller-Hinton broth (Difco) to give 108 colony-forming units/mL; the resuspended bacteria were then incubated.

4. Polymerase Chain Reaction (PCR) detection of *van A, B, C* genes

All VRE isolates were analysed for the presence of the vanA, vanB, vanC genes by multiplex PCR. Conditions of DNA isolation, PCR amplification and amplicon analysis by electrophoresis as well as primers and DNA controls used, have been described previously[13].

5. Detection of *mecA* gene and exotoxin gene

Detection of the mecA gene in the MRSA strains was performed by PCR amplication. For rapid extraction one to five bacterial colonies were suspended in 300 mL of cell lysis buffer and heated at 100℃ for 20 min. After centrifugation at 12,000 rpm for 10 min, 2 mL of the supernatant was used for the DNA extraction. PCR reactions were performed using a MRSA Primer Mix Kit (Genotek Co., Korea). The PCR amplification consisted of 30 cycles (94℃, 60 sec; 55℃, 60 sec; 72℃ 60 sec). The primers used in this study were as follows: *mecA* forward primer 5′-ATGAGATT AGGCATCGTTTC-3′, reverse primer; 5′-TGGATGACAGTACC TGAGCC-3′[14]. The primers for detection of enterotoxin gene were described in Table 1[15]. The final PCR products were separated on 2% agarose gel. ampicillin (AM) and gentamicin (GE) were commercially purchased from Sigma Chemical Co. (USA).

6. Determination of minimum inhibitory concentrations (MIC)

The MIC was performed by the microdilution broth method (CLSI, 2006). Serial two fold dilutions of AM, GM, 2S-DMDF, vancomycin was prepared in sterile 96-well micro plates and microtube with concentrations ranging MIC by using MHB. The MRSA and VRE suspensions were adjusted to the 0.5 McFarland standards (approximately 1×10^8 CFU/mL). Final inoculums were adjusted to the 10^6 CFU/mL. The MHB was supplemented with serial AM, GM and vancomycin concentrations ranging from 0.25 to 1024 μg/mL, and with 2S-DMDF at concentrations from 0.24 to 1,000 μg/mL. The data were reported as MICs, the lowest concentration of AM, GM and vancomycin inhibiting visible growth after 16 hr of

incubation at 37℃[16].

Results and Discussion

In general, MRSA strains show resistance against multiple antimicrobial agents although the ranges and extents of these resistances are versatile. In many years past, vancomycin was the only effective drug for serious MRSA infections[17]. The report provided a genetic analysis in that the vanA gene made MRSA highly resistant to vancomycin. VRE was not observed until the 1980s. During the 1990s, however, a drastic rise in VRE infections occurred[18,19]. Intrinsically, enterococci (especially E. faecium) possess a broad range of resistance against antimicrobials. Therefor, the demand for the development of new antibacterial agents effective against MRSA and/or VRE is a matter of great urgency. Thus, we have been trying to discover effective growth inhibitors against MRSA and/or VRE. There are several reports on natural anti-MRSA compounds, such as 3-arylcoumarines[20], licoricidin[21], cudraxanthone S[22], piperitylmagnolol[23] and isolupalbigenin[24]. Recently, several researchers have reported that these compounds have proven effective against tumors, arrhythmia, and immunodeficiency, and thus have generated a great deal of attention and interest[25-28]. Many researchers have focused on the matrine-type alkaloids and prenylated flavonoids[29], due to their quantitative and qualitative aspects.

In particular, there is growing interest in the prenylated flavonoids of S. flavescens, which are implicated in the antiinflammatory[30,31], anti-diabetic[32], monoamine oxidase inhibitory[33], and cytotoxic effects[34]. Among these flavonoids, sophoraflavanone G and kurarinone were reported to have potent cytotoxic activities[34,35], radical scavenging activities[31], as well as inhibitory activities against tyrosinase[36,37], α-glucosidase and β-amylase[32]. Also, kuraridin, kurarinone, and sophoraflavanone G were reported to possess cyclooxygenases and lipoxygenases inhibitory effects[30,38]. Previously, we demonstrated the antioxidant properties of 8-lavandulylkaempferol from S. flavescens, as a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and peroxynitrite scavenger in vitro[39].

The antimicrobial effects of 2S-DMDF on human pathogenic bacteria, including clinical isolates of antibiotic-resistant bacteria, were investigated and described as the MIC. Table 2 shows the MICs of AM, GM and 2S-DMDF against *S. aureus*. Of the 6 strains used in the present study, the standard strain of *S. aureus* ATCC 33591 and 5 clinical isolates were *mecA* positive. *S. aureus* ENT2, ENT3, ENT6, ENT9 and ENT19 are clinically isolated MRSA strains that showed very high MICs of Cephalothin, Clindamycin, Erythromycin, Oxacillin, Gentamicin, Penicillin(Table 1).

Table 1. MIC characteristics of ENT-MRSA isolates from patients with hearing loss.

Source of isolates (No. tested)	Antimicrobial agent	MIC (mg/mL)*		
		Rang	50%	90%
Patients(23)	Cephalothin	1- ≥128	128	128
	Clindamycin	≤0.5, ≥128	≥128	≥128
	Erythromycin	≤0.5 - ≥128	≥128	≥128
	Gentamicin	≤0.5 - ≥128	64	≥128
	Oxacillin	8 - ≥128	≥128	≥128
	Penicillin	≤0.5 - 128	64	128

*CLSI : Performance Standards for Antimicrobial Susceptibility Testing : Eighteenth

Against MRSA with enterotoxin gene, the MIC/MBC of AM 125-256 / 125-256 μg/mL, and the MIC/MBC of GM 64-512< / 64-512< μg/mL (Table 2). Thus, these antimicrobial agents are basically ineffective on these MRSA strains. So, we measured the MICs of 2S-DMDF with several MRSA strains. In this result, we observed MIC values of 0.97 to 15.6 mg/mL with the MRSA strains tested(Table 2). 2S-DMDF were able to inhibit the growth of these MRSA strains at relatively low concentrations. However, MICs of 2S-DMDF against MRSA with enterotoxin gene sec strains were a little higher than that of MRSA with other enteritoxin gene(Table 2).

Table 2. MIC/MBC of 2S-DMDF and ampicillin, gentamicin against ENT MRSA.

Strains	Agent	MIC (mg/mL)	MBC (mg/mL)
S.aureus (33592)	Ampicillin	256	256
	Gentamicin	512	512
	2S-DMDF*	0.97	1.95
ENT2 (TSST)	Ampicillin	256	256
	Gentamicin	256	256
	2S-DMDF	3.90	3.90
ENT3 (TSST,sec)	Ampicillin	256	256
	Gentamicin	256	256
	2S-DMDF	0.97	0.97
ENT6 (etb)	Ampicillin	256	256
	Gentamicin	256	256
	2S-DMDF	3.90	7.80
ENT9 (sec)	Ampicillin	125	125
	Gentamicin	256	512
	2S-DMDF	15.6	15.6
ENT19 (TSST,sea,sec)	Ampicillin	256	256
	Gentamicin	64	64
	2S-DMDF	1.95	3.90

*(2S)-7,4'-dihydroxy-5-methoxy-8-(γ, γ-dimethylallyl)-flavanone

Thus, 2S-DMDF are effective on MRSA at similar concentrations. Also, against VRE strains, the MIC of vancomycin was very high, higher than 512 mg/mL. And against VRE with vanA gene strains, the MIC/MBC for vancomycin were determined to be 512-1024</512-1024< mg/mL; for AM, 125-256/125-256 mg/mL. I also tested the

antibacterial activities of 2S-DMDF on VRE. I observed MIC values being 7.8 to 15.6 mg/mL for VRE strains, respectively, with 2S-DMDF(Table 3). Effect of antibacterial activity of 2S-DMDF in VRE exhibited less activity than MRSA(Table 3).

Table 3. MIC/MBC of 2S-DMDF and vancomycin VRE with *vanA* gene.

Strains	Agent	MIC (mg/mL)	MBC (mg/mL)
VRE24 (*vanA*)	Ampicillin	256	256
	Vancomycin	1024<	1024<
	2S-DMDF*	15.6	15.6
VRE189 (*vanA*)	Ampicillin	512	512
	Vancomycin	512	512
	2S-DMDF	7.8	7.8
VRE186 (*vanA*)	Ampicillin	125	256
	Vancomycin	512	512
	2S-DMDF	15.6	15.6
VRE233 (*vanA*)	Ampicillin	512	512
	Vancomycin	1024<	1024<
	2S-DMDF	7.8	7.8
VRE232 (*vanA*)	Ampicillin	256	256
	Vancomycin	512	512
	2S-DMDF	15.6	15.6
VRE238 (*vanA*)	Ampicillin	512	512
	Vancomycin	512	512
	2S-DMDF	15.6	15.6

*(2S)-7,4'-dihydroxy-5-methoxy-8-(γ, γ-dimethylally)-flavanone

Most would agree that extracellular toxins play a major role. As such, antibiotics that are capable of suppressing these toxins could provide added advantage in the treatment of these MRSA infections. In this result, my findings indicate that this compound was uniformly active against all strains of MRSA with enterotoxin gene and VRE with vanA gene.

Further studies with large sample scales needed for the statistical correlation significance of MRSA with enterotoxin gene from clinical speciemens. It is suggest that the results cannot be applied currently in clinical treatment, but consider that the 2S-DMDF will prove to be helpful to treat MRSA.

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References

1. Rice, L.B. Antimicrobial resistance in gram-positive bacteria. Am J Infect Control. 34: S11-S9, 2006.
2. Fujita, N. Vancomycin-resistant Enterococci (VRE)--for VRE endemics in apan. Rinsho Biseibutshu Jinsoku Shindan Kenkyukai Shi. 16: 1-16, 2005.
3. Heslop, A., Ovesen, T. Severe acute middle ear infections: microbiology and treatment. Int J Pediatr Otorhinolaryngol. 70: 1811-1816, 2006.
4. Pajor, A., Durko, M., Jankowski, A., Stańczyk, A. Bartoszko-Tyczkowska, Bacteriological evaluation in chronic otitis media. Otolaryngol Pol. 60: 757-763, 2006.
5. da Cunha Mde, L., Calsolari, R.A., Júnior, J.P. Detection of enterotoxin and toxic shock syndrome toxin 1 genes in Staphylococcus, with emphasis on coagulase-negative staphylococci. Microbiol Immunol. 51: 381-390, 2007.
6. Rosec, J.P., Guiraud, J.P., Dalet, C., Richard, N. Enterotoxin production by staphylococci isolated from foods in France. Int J Food Microbiol. 35: 213-221, 1997.
7. Brewer, J.D., Hundley, M.D., Meves, A., Hargreaves, J., McEvoy, M.T., Pittelkow, M.R. Staphylococcal scalded skin syndrome and toxic shock syndrome after tooth extraction. J Am Acad Dermatol. 59: 342-346, 2008.
8. Schlievert, P.M., Shands, K.N., Dan, B.B., Schmid, G.P., Nishimura, R.D. Identification and characterization of an exotoxin from *Staphylococcus aureus* associated with toxic-shock syndrome. J Infect Dis. 143: 509-516, 1981.
9. Linton, A.H., Hedges, A.J., Bennett, P.M. Monitoring for the development of antimicrobial resistance during the use of olaquindox as a feed additive on commercial pig farms. J Appl Bacteriol. 64: 311-327, 1988.
10. Botta, B., Vitali, A., Menendez, P., Misiti, D., Delle Monache, G. Prenylated flavonoids: pharmacology and biotechnology. Curr Med Chem. 12: 717-739, 2005.
11. Kang, S.S., Kim, J.S., Son, K.H., Chang, H.W., Kim, H.P. A new prenylated flavanone from the roots of Sophora flavescens. Fitoterapia, 71: 511-515, 2000.
12. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard 9th ed. Document M100-S19. Clinical and Laboratory Standards Institute, Villanova, Pa, USA, 2009.
13. Coque, T.M., Tomayko, J.F., Ricke, S.C., Okhyusen, P.C., Murray, B.E. Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. Antimicrob Agents Chemother. 40: 2605-2609, 1996.
14. Ryffel, C., Tesch, W., Birch-Machin, I., Reynolds, P.E., Barberis-Maino, L., Kayser, F.H., Berger-Bächi, B. Sequence comparison of mecA genes isolated from methicillin-resistant *Staphylococcus aureus* and Staphylococcus epidermidis. Gene. 28: 137-138, 1990.
15. Becker, K., Roth, R., Peters, G. Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and

hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. J Clin Microbiol. 36: 2548-2553, 1998.

16. Shahverdi, A.R., Fakhimi, A., Zarrini, G., Dehghan, G., Iranshahi, M. Galbanic acid from Ferula szowitsiana enhanced the antibacterial activity of penicillin G and cephalexin against *Staphylococcus aureus*. Biol Pharm Bull. 30: 1805-1807, 2007.
17. Aucken, H.M., Ganner, M., Murchan, S., Cookson, B.D., Johnson, A.P. A new UK strain of epidemic methicillin-resistant *Staphylococcus aureus* (EMRSA-17) resistant to multiple antibiotics. J Antimicrob Chemother. 50: 171-175, 2002.
18. Tsiodras, S., Gold, H.S., Sakoulas, G., Eliopoulos, G.M., Wennersten, C., Venkataraman, L., Moellering, R.C., Ferraro, M.J. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet. 358: 207-208, 2001.
19. Toh, S.M., Xiong, L., Arias, C.A., Villegas, M.V., Lolans, K., Quinn, J., Mankin, A.S. Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant *Staphylococcus aureus* resistant to the synthetic antibiotic linezolid. Mol Microbiol. 64: 1506-1514, 2007.
20. Hatano, T., Shintani, Y., Aga, Y., Shiota, S., Tsuchiya, T., Yoshida, T. Phenolic constituents of licorice. VIII. Structures of glicophenone and glicoisoflavanone, and effects of licorice phenolics on methicillin-resistant *Staphylococcus aureus*. Chem Pharm Bull (Tokyo). 48: 1286-1292, 2000.
21. Fukai, T., Marumo, A., Kaitou, K., Kanda, T., Terada, S., Nomura, T. Antimicrobial activity of licorice flavonoids against methicillin-resistant *Staphylococcus aureus*. Fitoterapia. 73: 536-539, 2002.
22. Fukai, T., Oku, Y., Hou, A.J., Yonekawa, M., Terada, S. Antimicrobial activity of hydrophobic xanthones from Cudrania cochinchinensis against Bacillus subtilis and methicillin-resistant *Staphylococcus aureus*. Chem Biodivers. 1:1385-1390, 2004.
23. Syu, W.J., Shen, C.C., Lu, J.J., Lee, G.H., Sun, C.M. Antimicrobial and cytotoxic activities of neolignans from Magnolia officinalis. Chem Biodivers. 1: 530-537, 2004.
24. Sato, M., Tanaka, H., Tani, N., Nagayama, M., Yamaguchi, R. Different antibacterial actions of isoflavones isolated from Erythrina poeppigiana against methicillin-resistant *Staphylococcus aureus*. Lett Appl Microbiol. 43: 243-248, 2006.
25. Kang, T.H., Jeong, S.J., Ko, W.G., Kim, N.Y., Lee, B.H., Inagaki, M., Miyamoto, T., Higuchi, R., Kim, Y.C. Cytotoxic lavandulyl flavanones from Sophora flavescens. J Nat Prod. 63: 680-681, 2000.
26. Dai, S., Chan, M.Y., Lee, S.S., Ogle, C.W. The antiarrhythmic effects of Sophora flavescens Ait. in rats and mice. Am J Chin Med. 14: 119-123, 1986.
27. Xiang, Q., Tan, M.Q., Huang, Y.H. Anti-leukemia effect of sophora flavescens combined with the low molecular weight natural tumor suppressor of the human fetal liver and its mechanism. Hunan Yi. Ke Da Xue Xue Bao. 27: 108-110, 2002.
28. Sun, M., Han, J., Duan, J., Cui, Y., Wang, T., Zhang, W., Liu, W., Hong, J., Yao, M., Xiong, S., Yan, X. Novel antitumor activities of Kushen flavonoids in vitro and in vivo. Phytother Res. 21: 269-277, 2007.
29. Zhang, L., Xu, L., Xiao, S.S., Liao, Q.F., Li, Q., Liang, J., Chen, X.H., Bi, K.S. Characterization of flavonoids in the extract of Sophora flavescens Ait. by high-performance liquid chromatography coupled with diode-array detector and electrospray ionization mass spectrometry. J Pharm Biomed Anal. 44: 1019-1028, 2007.
30. Kim, D.W., Chi, Y.S., Son, K.H., Chang, H.W., Kim, J.S., Kang, S.S., Kim, H.P. Effects of sophoraflavanone G, a prenylated flavonoid from Sophora flavescens, on cyclooxygenase-2 and in vivo inflammatory response. Arch Pharm Res. 25: 329-335, 2002.
31. Piao, X.L., Piao, X.S., Kim, S.W., Park, J.H., Kim, H.Y., Cai, S.Q. Identification and characterization of antioxidants from Sophora flavescens. Biol Pharm Bull. 29: 1911-1915, 2006.
32. Kim, J.H., Ryu, Y.B., Kang, N.S., Lee, B.W., Heo, J.S., Jeong, I.Y., Park, K.H. Glycosidase inhibitory flavonoids from Sophora flavescens. Biol Pharm Bull. 29: 302-305 2006.
33. Hwang, J.S., Lee, S.A., Hong, S.S., Lee, K.S., Lee, M.K., Hwang, B.Y., Ro, J.S. Monoamine oxidase inhibitory components from the roots of Sophora flavescens. Arch Pharm Res. 28: 190-194, 2005.
34. Kang, T.H., Jeong, S.J., Ko, W.G., Kim, N.Y., Lee, B.H., Inagaki, M., Miyamoto, T., Higuchi, R., Kim, Y.C. Cytotoxic lavandulyl flavanones from Sophora flavescens. J Nat Prod. 63: 680-681, 2000.
35. De Naeyer, A., Vanden Berghe, W., Pocock, V., Milligan, S., Haegeman, G., De Keukeleire, D. Estrogenic and anticarcinogenic properties of kurarinone, a lavandulyl flavanone from the roots of Sophora flavescens. J Nat Prod. 67: 1829-1832, 2004.
36. Son, J.K., Park, J.S., Kim, J.A., Kim, Y., Chung, S.R., Lee, S.H. Prenylated flavonoids from the roots of Sophora flavescens with tyrosinase inhibitory activity. Planta Med. 69: 559-561, 2003.

37. Kim, S.J., Son, K.H., Chang, H.W., Kang, S.S., Kim, H.P. Tyrosinase inhibitory prenylated flavonoids from Sophora flavescens. Biol Pharm Bull. 26: 1348-1350, 2003.
38. Chi, Y.S., Jong, H.G., Son, K.H., Chang, H.W., Kang, S.S., Kim, H.P. Effects of naturally occurring prenylated flavonoids on enzymes metabolizing arachidonic acid: cyclooxygenases and lipoxygenases. Biochem Pharmacol. 62: 1185-1191, 2001.
39. Jung, H.J., Kang, S.S., Woo, J.J., Choi, J.S. A new lavandulylated flavonoid with free radical and ONOO- scavenging activities from Sophora flavescens. Arch Pharm Res. 28: 1333-1336, 2005.